NITRIFICATION ENHANCEMENT THROUGH pH CONTROL WITH ROTATING BIOLOGICAL CONTACTORS

FINAL REPORT

by

James M. Stratta
David A. Long

Civil Engineering Department
The Pennsylvania State University

September, 1981

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-79-C-9110

Institute for Research on Land and Water Resources
The Pennsylvania State University

Approved for public release, distribution unlimited.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER

2. GOVT ACCESSION NO. AD-A104 420

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)

NITRIFICATION ENHANCEMENT THROUGH pH CONTROL WITH ROTATING BIOLOGICAL CONTACTORS

5. TYPE OF REPORT & PERIOD COVERED
Final-- 1 August 79-
31 July 81

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)

James Stratta - US Army
David A. Long - The Pennsylvania State University

8. CONTRACT OR GRANT NUMBER(s)
DAMD17-79-C-9110

9. PERFORMING ORGANIZATION NAME AND ADDRESS

Institute for Research on Land and Water Resources
The Pennsylvania State University
University Park, PA 16802

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
62720A.3E162720A835.00.092

11. CONTROLLING OFFICE NAME AND ADDRESS
US Army Medical Research and Development Command
Fort Detrick
Frederick, MD 21701

12. REPORT DATE
September, 1981

13. NUMBER OF PAGES
167

14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)
US Army Medical Bioengineering and Research Laboratory
Fort Detrick
Frederick, MD 21701

15. SECURITY CLASS. (of this report)
Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public; distribution unlimited.

Accession For
NTIS GRA&I X
DTIC TAB
Unannounced
Justification
By
Distribution/
Availability Codes
Dist | Avail and/or Special
A

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES
None

"Original contains color plates: All DTIC reproductions will be in black and white"

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Rotating Biological Contactors
Nitrification
pH Control
Ammonia
Alkaline Chemical
Biofilm
Nitrifying bacteria
Heterotrophic bacteria
Engineering Design
Alkalinity
Fixed film Systems

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The short and long term effect of pH on the nitrification of domestic wastewater with pilot scale RBC systems are examined. Alternative alkaline chemicals are evaluated for their ability to enhance the nitrification rate. Findings demonstrate greater nitrification rates at elevated pH levels and similar nitrification rates for various alkaline chemicals. Lime usage increases suspended solids which results in a need for additional settling prior to discharge. Design recommendations are included.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified 420420

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ABSTRACT

The recent use of rotating biological contactor (RBC) systems to nitrify low pH-low alkalinity domestic wastewaters has demonstrated the need for additional engineering design information regarding the long term effect of pH on nitrification and pH control methods to enhance the rate of nitrification. This research was designed to: (1) establish the relative rates of nitrification for an acclimated RBC fixed film as a function of pH; (2) characterize the fixed films with respect to rate of biofilm development as well as the number of ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, and heterotrophic bacteria; (3) evaluate alternative pH adjustment schemes utilizing a variety of alkaline chemicals; and (4) develop RBC design recommendations.

Phase I research was devoted to evaluating the long term effect of pH on nitrification. The relative effectiveness of four different alkaline chemicals on enhancing the nitrifying process under optimum pH conditions was evaluated in Phase II. Pilot scale 0.5 meter diameter RBC systems were used to nitrify high rate trickling filter effluent with chemical control of pH and alkalinity.

Phase I research demonstrated the following: (1) in the long term, distinctly higher nitrification rates are associated with increasing pH up to a maximum of pH 8.5; (2) RBC nitrifying biofilms mature in several weeks as evidenced by heavier biofilms and more uniform disc coverage associated with increasing pH up to pH 8.5; (3) ammonia stripping and denitrification did not play a significant role in the ammonia removal process; and (4) biofilms developed at elevated

pH levels retain their high degree of nitrification for one to three weeks following a sudden and permanent downward shift in pH. In Phase II, the ammonia removal efficiencies for five 2-stage RBC systems were 87, 87, 86, 82, and 73 percent for the lime, sodium carbonate, caustic, sodium bicarbonate, and the low pH control RBC, respectively. Lime addition resulted in excessive suspended solids buildup within the wastewater requiring clarification following the nitrification step. Based upon the biofilm and bacterial studies in both research phases, the following conclusions were drawn regarding the characteristics of the nitrifying RBC biofilms: (1) the ratio of heterotrophic bacteria to ammonia-oxidizing bacteria to nitrite-oxidizing bacteria is approximately 10:10:1; (2) the ratio of ammonia-oxidizers to nitrite-oxidizers increases with increasing pH; and (3) long term biofilm development is a function of pH with the heaviest and most uniform disc coverage occurring with increasing pH up to pH 8.5.

This fixed film research effort evaluated the effect of pH on nitrification for a longer period of time than any previous suspended or fixed film study which has been reported. The results bring together the salient and diverse findings of previous research on the subject. Design recommendations are presented which will enable engineers to optimize the use of RBC systems for ammonia removal.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

Alk.	alkalinity
CBOD	carbonaceous biochemical oxygen demand, 5 day value
cm	centimeter
CO_2	carbon dioxide
DO	dissolved oxygen
dvg	dry volatile gram
g	gram
$gal/d \cdot ft^2$	gallons per day per square foot
l	liter
m	meter
m^3/d	cubic meters per day
MGD	millions of gallons per day
$m^3/d \cdot m^2$	cubic meters per day per square foot
mg/cm^2	milligrams per square centimeter
mg/l	milligrams per liter
ml	milliliter
MPN	most probable number
NH_3-N	ammonia-nitrogen
NO_2-N	nitrite-nitrogen
NO_3-N	nitrate-nitrogen
$(NO_2 + NO_3)$-N	nitrite plus nitrate nitrogen
NPDES	National Pollution Discharge Elimination System
OP-P	orthophosphate-phosphorus
Org-N	organic nitrogen
pH	negative log of hydrogen ion concentration

ABBREVIATIONS AND SYMBOLS

pmole	picomole
PSU	Pennsylvania State University
RBC	rotating biological contactor
rpm	revolutions per minute
scm	square centimeter
SS	suspended solids
std. dev.	standard deviation
TKN-N	total Kjeldahl nitrogen
TP-P	total phosphate-phosphorus
VSS	volatile suspended solids
WWTP	wastewater treatment plant
^{o}C	degrees Celsius

FOREWORD

The authors would like to express their appreciation to Ms. Dama Wirries and the laboratory staff of the Pennsylvania State Institute for Research on Land and Water Resources for physical and chemical wastewater analysis associated with this study. Appreciation also is expressed to Mr. Michael C. Doherty for performing microbial enumerations and assistance in operating the pilot plant facilities. This research was performed for the United States Army Medical Research and Development Command under contract No. DAMD17-79-C-9110. RBC research facilities were provided by Autotrol Corporation, Milwaukee, Wi.

Citations for organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

Personnel supported by the contract included: Dr. David A. Long, Associate Professor of Civil Engineering; Ms. Dama L. Wirries, Senior Project Associate and Mr. Michael C. Doherty, Graduate Assistant, all of The Pennsylvania State University.

Publications supported by the contract included:

Stratta, J. M. and Long, D. A., "Nitrification Enhancement through pH Control with Rotating Biological Contactors," Proceedings First National Symposium/Workshop on Rotating Biological Contactor Technology, Vol. II, Champion, Pa., February, 1980.

Stratta, J. M., "Nitrification Enhancement Through pH Control with Rotating Biological Contactors," Ph.D. Thesis, The Pennsylvania State University, August 1981.

Doherty, M. C., "Heterotrophic Bacterial Population in a Rotating Biological Contactor Nitrification System," M.E.P.C. Paper, The Pennsylvania State University, August 1981.

SECTION I

INTRODUCTION

1.1 Background

1.1.1 RBC Technology - General

The rotating biological contactor (RBC) is the currently accepted generic term used to describe a wastewater treatment system composed of closely spaced discs mounted on a horizontal shaft and rotated while submerged approximately 40 percent in wastewater. The discs provide a fixed surface area for the attachment of bacteria which biologically degrade the wastewater. The forerunner of the RBC initially was developed about 1900 in Germany where a patent was issued for a rotating cylinder made up of wooden slats. In 1929, a "biological wheel" consisting of rotating paddle wheels was developed in the United States. The first use of rotating discs for the contact media occurred also in the United States in 1929. This early application utilized metal discs, but without promising results. It was not until plastic media discs were tested successfully at Stuttgart University in Germany in the late 1950s that RBC technology was put on a commercial footing. The first commercial RBC wastewater treatment system was installed in Germany in 1960 and its application spread quickly throughout Europe. Most of these early RBC installations serviced less than 1000 people. The development of RBC technology by Allis-Chalmers in the United States progressed independently of the work in Europe. Autotrol Corporation purchased Allis-Chalmers in 1970 and in 1972 developed a corrugated polyethylene contactor media which vastly increased the surface area available per unit volume of contactor media. This innovation

reduced capital construction costs and was an important factor in the commercial acceptance of RBC wastewater treatment systems in the United States (7).

There are many advantages associated with the treatment of wastewater with RBCs. These advantages include: (1) relatively high treatment efficiency, (2) low power and maintenance costs, (3) high oxygen transfer, (4) high retrofit potential, (5) simplicity of operation, (6) resistance to biofilm washout, (7) reduced odors, (8) no short circuiting, and (9) no bulking or foaming problems to interfere with efficiency (85). The high degree of treatment, low power cost, and a vigorous marketing campaign by the RBC industry have been responsible for the relatively rapid spread of RBC systems in the United States (54).

There are more than 260 RBC installations providing secondary treatment for municipal wastewater in the United States today. Most of these RBC installations are small systems treating less than 19,000 m^3/d (5 MGD) of domestic wastewater. An examination of the first few years of experience with full scale RBCs in the United States has identified certain problems associated with their use. These problem areas include: (1) frequent inability to perform up to design expectations, (2) shaft, media, and bearing failures, (3) lack of process controls, (4) noneconomy of scale, (5) land area requirements proportional to plant capacity, (6) solids accumulation in the troughs, (7) growth of nuisance bacteria, such as Beggiatoa, on the discs, and (8) problems in design associated with scale-up from pilot plant operations (20, 85). Some of the physical plant problems are due to the rapid growth of this relatively new wastewater treatment industry. These problems should be resolved as more operational experience is gathered. The frequent

inability to perform up to design expectations is due, in part, to early process performance analysis being carried out by the RBC industry without independent corraboration. Better design information is being made available through the proliferation of independent RBC research in recent years.

1.1.2 Nitrification with RBCs

The need to achieve compliance with ammonia-nitrogen discharge limitations and the current emphasis on energy conservation has resulted in the utilization of RBC technology for the nitrification of secondary wastewater effluents. By the end of the 1970s, four pilot scale efforts, independent of the RBC industry, had been completed which demonstrated that the RBC could nitrify successfully secondary wastewater effluent (16, 41, 71, 76). In 1979, approximately 70 percent of the RBC systems in the United States were designed to remove carbonaceous biochemical oxygen demand (CBOD). Another 25 percent of the RBC systems were designed to remove CBOD and for nitrification. The remaining 5 percent were constructed to nitrify secondary wastewater effluents in order to achieve ammonia-nitrogen effluent discharge limitations (49). Initial evaluations of full scale nitrifying RBC facilities reveal that they have not been completely satisfactory (22, 42). Hitdlebaugh (42) reported that an RBC facility, built for CBOD removal and nitrification, failed to meet design specifications during both winter and summer operations. The inability to meet CBOD and ammonia-nitrogen limitations during the summer was attributed to relatively low dissolved oxygen (DO) concentrations (less than 1 mg/1) in the initial nitrifying stages and a low pH (less than pH 7.0) in the latter nitrifying stages. The DO level increased during winter

operations and CBOD was removed sufficiently to achieve design expectations. Ammonia-nitrogen removal also improved during the winter but not sufficiently to achieve design projections or effluent limitations. Recommendations from this study included the use of alkaline chemical feed systems to maintain optimum pH levels in order to improve nitrification.

1.1.3 Nitrification, pH and Alkalinity

Nitrification within the RBC biofilm is essentially a two step microbiological process which utilizes two groups of autotrophic bacteria of the family Nitrobacteraceae. The first group of bacteria oxidizes ammonia to nitrite and the second group of bacteria oxidizes nitrite to nitrate. The currently recognized genera of nitrifying bacteria are listed in Table 1.1. This listing was prepared by Belser (13) and is an update of the nitrifying organisms described in Bergy's Manual (102). The Nitrosomonas and Nitrobacter genera, listed in Table 1.1, are considered to be the predominant nitrifying bacteria inhabiting the wastewater environment. The other genera listed are thought to be more closely associated with soil and marine environments. Heterotrophic nitrification also occurs when nitrite or nitrate is produced from organic or inorganic compounds by heterotrophic organisms. Over 100 heterotrophic species (including fungi) have been identified which are capable of heterotrophic nitrification. However, the overall contribution to the oxidized nitrogen forms by heterotrophic nitrification is considered to be relatively small (79).

The growth rates of nitrifying bacteria are much slower than the growth rates of heterotrophic bacteria. This important distinction accounts for the inability of nitrification to proceed simultaneously

Table 1.1 Genera and Species of Autotrophic Nitrifying Bacteria[a]

Energy Sources	Genera	Species
Ammonium	Nitrosomonas	europaea
	Nitrosospira	briensis
	Nitrosococcus	nitrosus
		oceanus
		mobilus
	Nitrosolobus	multiformis
	Nitrosovibrio	tenuis
Nitrite	Nitrobacter	winogradskyi
	Nitrospina	gracilis
	Nitrococcus	mobilis

[a]From Belser (13, p 311).

with CBOD removal when high concentrations of organic material (greater than 30 mg/l of BOD) are present in the wastewater. Minimum doubling times reported for the ammonia-oxidizing bacteria are from 8 to 17 hours (78). Because the growth rates for nitrite-oxidizers are greater than the growth rates for ammonia-oxidizers (78, 97), elevated nitrite concentrations normally do not persist and the ammonia-oxidation step controls the total amount of ammonia which is oxidized to nitrate within the wastewater environment. Carbon dioxide (CO_2) is the carbon source for these autotrophic nitrifying bacteria (102). Although some nitrifying bacteria have been observed to use organic compounds, they were not observed to utilize these organic compounds as

the sole carbon source for growth (24). The generation of bacterial biomass per unit of ammonia oxidized, or yield, is quite small. The total yield for both Nitrosomonas and Nitrobacter has been observed to be from 0.06 to 0.20 gram of cells per gram of ammonia oxidized (78). The nitrification of 20 mg/l of ammonia-nitrogen generates approximately 2 mg/l of solids (97). The net amount of inorganic carbon required for this amount of nitrification is therefore quite low. McGee (67) reported that the inorganic carbon requirements for the nitrite oxidation step could be met without inorganic carbon in the bulk solution. The source of inorganic carbon was attributed to the CO_2 generated from endogenous respiration within the biofilm.

Initial operational experiences with nitrifying RBCs have demonstrated that the nitrification of domestic wastewater produces only low level increases in suspended solids so that the wastewater meets NPDES suspended solids discharge limitations without additional clarification (8, 9).

The level of DO also is an important consideration within the RBC nitrification process. Minimum levels of DO needed for nitrification, which have been reported in the literature, vary from 0.5 to 4 mg/l (7, 78, 97). In general, DO levels greater than 1-2 mg/l have resulted in satisfactory rates of nitrification. The rate of disc rotation is a key design parameter affecting the rate of oxygen transfer as well as the diffusion of substrate into the biofilm (57). A disc peripheral velocity of approximately 0.3 m/sec has been reported in many studies as having produced adequate DO levels for nitrification.

Temperature is an important factor which affects the rate of nitrification. Huang (47) reports on 20 papers which address the effect

of temperature. The effect of temperature on nitrification within the RBC is not clearly defined (7). The best available data, based upon full scale RBC data (11), reveals that between 5°C and 18°C, the effect of wastewater temperature is to double the nitrification rate for each 10°C increase. RBC systems are designed with sufficient surface area to meet both summer and winter conditions. Fortunately, many NPDES discharge limitations are less stringent in the colder winter months. This procedure reduces the total RBC surface area required in the design, because winter temperature surface area requirements would govern under the more stringent limitations.

Current RBC designs for the nitrification of wastewater are based upon hydraulic loading. Recent research efforts have addressed design loadings ranging up to approximately 200 $l/m^2 \cdot d$ (5 $gal/d \cdot ft^2$) (16, 41, 71, 76). These findings reveal that the nitrification efficiency decreases as the hydraulic loading increases. Full scale designs of nitrifying RBCs are generally in the range of 60 to 80 $l/m^2 \cdot d$) (approximately 1.2 to 1.6 g NH_3-$N/m^2 \cdot d$).

The RBC nitrification kinetics have not been defined fully although the subject has been addressed by a number of investigators. In general, the ammonia removal process can be described as a first order reaction with respect to staging, or RBC detention time. More specifically, the amount of ammonia remaining as a function of progressive RBC stages, decreases according to a first order relationship (7). The rate of ammonia oxidation within a stage is reported to follow zero order to first order kinetics (7, 8, 46, 74, 81).

The simplified oxidative reactions below describe the salient aspects of the microbial oxidation of ammonia. The microorganisms

derive energy from these reactions, this energy is used for CO_2 fixation.

Ammonia Oxidation:

$$NH_4^+ + 1.5O_2 + 2HCO_3^- \longrightarrow NO_2^- + 2H_2CO_3 + H_2O \qquad (1)$$

Nitrite Oxidation:

$$NO_2^- + 0.5O_2 \longrightarrow NO_3^- \qquad (2)$$

Overall Reaction:

$$NH_4^+ + 2O_2 + 2\,HCO_3^- \longrightarrow NO_3^- + 2H_2CO_3 + H_2O \qquad (3)$$

As can be seen from Equation 1, the nitrification process results in the production of acid which neutralizes the alkalinity in the wastewater. Theoretically, 7.1 mg/l of alkalinity are destroyed for each 1 mg/l of ammonia oxidized. The destruction of alkalinity results in pH depression. The actual pH depression is mitigated somewhat by the removal of carbonic acid through the stripping of CO_2 from the wastewater surface (97). However, under low alkalinity conditions, pH depression is enhanced due to the reduced buffer capacity of the wastewater. The level of alkalinity within wastewaters varies widely. The major factor influencing the amount of alkalinity present is the carriage water, or the drinking water supply. High alkalinities normally are associated with ground water supplies and much lower alkalinities are associated with surface supplies. Domestic wastewater contributes from 50 to 200 mg $CaCO_3$/l to the natural alkalinity of the carriage water (69). Therefore, the amount of alkalinity in a domestic wastewater may range from less than 100 mg $CaCO_3$/l to several hundred mg $CaCO_3$/l. The net effect of such variations in alkalinity is to provide a different buffering capacity for each wastewater treatment system. Domestic wastewaters normally contain from 12 to 25 mg/l

of ammonia-nitrogen. The range of alkalinity destroyed during the nitrification of these concentrations of ammonia is 85 mg $CaCO_3$/l to 178 mg $CaCO_3$/l. Obviously, the pH depression can be slight for low ammonia-high alkalinity wastewaters or significant for high ammonia-low alkalinity wastewaters. Wastewater pH levels are typically around pH 7.5. However, pH depression to below pH 7.0 is common for low alkalinity wastewaters. Sufficient pH depression has been observed in the nitrification of domestic wastewater to violate NPDES discharge limitations solely on the basis of pH (91).

The level of pH has an important effect on the nitrification process. There have been a number of researchers since the turn of the century who have addressed the subject of the effect of pH on nitrification. Those researchers who have made contributions pertinent to this study are listed in reverse chronological order in Table 1.2. It is interesting to note the large variation in the effect of pH on biological nitrification that is reported in the literature. The variation in the effect of pH on nitrification is due in large measure to the nature of the experiments undertaken, i.e., the homogeniety of culture involved (pure versus mixed culture); scale of the experiment (laboratory to full scale); nature of the biofilm (suspended versus fixed film) and a variety of (and frequently unspecified) acclimation times utilized in the experiments.

Several investigators (16, 39, 47, 71) within recent years have attempted to provide more information specifically with regard to nitrification within a fixed film mode. Haug and McCarty (39) utilized a laboratory scale fixed film submerged reactor and a synthetic wastewater and performed a short term pH-nitrification study (18 hours at

Table 1.2 Literature Review of Optimum pH Values for Nitrification

Ref.	Author	Year	Optimum pH	Organism or System Studied
71	Miller, et al.	1979	8.0-8.5	RBC Biofilm (Pilot)
16	Borchardt, et al.	1978	7.1-8.6	RBC Biofilm (Pilot)
67	McGhee	1975	8.0-9.0	Activated Sludge (Lab.)
87	Srna and Baggaley	1975	7.45	Submerged Filter Biofilm (Lab.)
48	Hutton and LaRocca	1975	8.4-8.6	Activated Sludge
47	Huang and Hopson	1974	8.4-9.0	Inclined Biofilm (Lab.)
39	Haug and McCarty	1972	7.8-8.3	Submerged Filter Biofilm (Lab.)
73	Mulbarger	1972	8.4	Activated Sludge
104	Wild, et al.	1971	8.4	Activated Sludge (Lab.)
62	Loveless and Painter	1968	7.5-8.0	Nitrosomonas
28	Downing and Knowles	1967	7.2-8.0	--
3,4	Andersen	1964	8.4-8.5	Nitrosomonas
15	Boon and Landelot	1962	7.0-8.6	Nitrobacter
31	Engel and Alexander	1958	7.0-9.0	Nitrosomonas
18	Bushwell and Shiota	1954	8.0-8.5	Nitrosomonas
45	Hoffman and Lees	1952	8.0-9.0	Nitrosomonas
70	Meyerhoff	1917	8.5-8.8	Nitrosomonas
			8.5-9.0	Nitrobacter

each pH value) using a biofilm developed at neutral pH and observed essentially the same rate of nitrification at pH 6.5 as at pH 9.0. At pH 6.0, the observed rate of nitrification was reduced to approximately 42 percent of the maximum rate and nitrification essentially stopped at

pH 5.5. However, after only 10 days of operation at pH 6.0, the submerged filter was reported to have acclimated sufficiently to perform at the maximum rate of nitrification. This finding demonstrates the ability of nitrifying organisms to acclimate to low pH conditions; unfortunately, the ability of nitrifying organisms to perform equally well at pH 6.0 as at pH 8.5 has never been shown by any other researcher. The reason for this unique finding may be due to non-equilibrium conditions existing within the submerged filter after the startup period. Huang and Hopson (47) utilized a laboratory scale inclined fixed film surface and a synthetic wastewater to evaluate the effect of pH on nitrification. Their experiment examined the short term (less than 10 hours) effect of pH on the nitrification process and produced a maximum rate of nitrification at pH 8.4 to pH 9.0 with approximately 25 percent of the maximum rate occurring at pH 6.0. After three weeks of acclimation at pH 6.6, the rate of nitrification was approximately 85 percent of the maximum rate observed.

Borchardt (16) performed a short term pH-nitrification study utilizing a 0.6 meter pilot RBC treating domestic wastewater effluent from a trickling filter in a laboratory where ammonia, alkalinity and pH were controlled. The rate of nitrification was examined at eleven different levels of alkalinity after a short but undefined acclimation period. The results of this short term study revealed a nearly constant rate of nitrification between pH 7.1 and 8.6 (no data were obtained at higher pH levels). Approximately 25 percent of the maximum rate of nitrification was observed at pH 6.5 and zero nitrification was indicated at pH 6.0. Borchardt, unlike many of his predecessors, was

careful to point out the limitations of attempting to extrapolate his short term data into the long term.

Miller (71) most recently reported on a pilot scale 0.5 meter RBC treating domestic wastewater effluent from a pilot trickling filter wherein significantly greater rates of nitrification were observed at elevated pH levels (pH 8.0 to pH 8.5) than at neutral pH (approximately pH 7.1). This nitrification study is unique in that lime addition for phosphorus removal preceded the nitrification process and the nitrifying RBC stages had acclimated at the elevated pH levels. A transition in biofilm performance was observed when the elevated pH of the wastewater was reduced to the neutral pH range. Nitrification performance initially remained unchanged. After approximately four days, the performance level started to deteriorate. In nine days, the performance had reverted to a lower nitrification level. This latter finding was not discussed fully by Miller; however, it is important because it helps to establish potential physical differences between the biofilms developed at neutral and elevated pH levels. This situation was not observed by the other investigators using fixed films mentioned above because none ever attempted to acclimate biofilms at the elevated pH levels. Such differences cannot be assumed to be purely indicative of only the pH dependent rates of microbial nitrification. These differences also are reflective of the entire heterogenous population developed within each biofilm which dictate film development, cohesion, and retention characteristics (sludge age). There is essentially no information within these wastewater nitrification studies which addresses changes in biofilm and microbial populations under various pH conditions. In general, this important consideration has been ignored in

such wastewater research studies. However, current research efforts such as those by Olem (75), LaMotta (61) and Characklis (19), are starting to examine more closely the mechanics of biofilm development and the characterization of microbial populations. Table 1.3 contains a listing of microbial enumerations of nitrifying and heterotrophic bacteria in a variety of nitrifying environments. These data demonstrate a wide variation in bacterial populations. These variations are due to differences in dispersion techniques, enumeration techniques, growth media, and incubation times as well as the inherent nature of the respective environments. These enumerations reveal that the heterotrophic population is a major element of any nitrifying biofilm and that the number of ammonia-oxidizing bacteria is generally much greater than the number of nitrite-oxidizing bacteria.

The addition of alkaline chemicals to wastewater treatment systems to increase pH and provide added buffer capacity has been attempted with varying degrees of success. Heidman (40) conducted a pilot study at the Blue Plains WWTP using an activated sludge system which incorporated pH controlled nitrification. This study was inconclusive because it failed to demonstrate the relative nitrification without chemical addition. Hutton (48) demonstrated the feasibility of optimizing the nitrification of high ammonia strength industrial wastewaters with alkaline chemical addition. Lue-Ling (63) reported success in using alkaline chemical addition to nitrify high ammonia strength lagoon supernatant with RBCs. Hitdlebaugh (43) attempted to enhance the nitrification of domestic wastewater with RBCs through alkaline chemical addition; however, the results were inconclusive. The literature fails to address the efficacy of optimizing domestic wastewater

Table 1.3 Microbial Enumerations[a] Within Nitrifying Environments

Reference	Environment	Number of Bacteria per Dry Milligram		
		Ammonia-Oxidizing	Nitrite-Oxidizing	Heterotrophic
Rowe (81)	Soil	1.2 - 2.0	-	-
LaBeda (60)	Soil	2.3	4,300	40,000,000
Matulewich (64)	River Sediment	462	16	-
Matulewich (65)	River Sediment	183	77	-
Matulewich (65)	River Rock Biofilm	36,200	143	-
Strom (90)	WWTP Influent	31,700	1,460	-
Strom (90)	WWTP Effluent	121,000	11,800	-
Ito (50)	Nitrifying RBC	20,000	2,000	2,000,000
Kaltreidger (55)	Activated Sludge	-	-	12,000,000
Davis (23)	Activated Sludge	15,000	2,500	180,000,000

[a]Enumerations are extracted from the cited literature. Appropriate assumptions were made, where necessary, to allow comparison of enumerations on a common basis.

nitrification within the RBC system through pH control as well as the use of alternative pH control schemes.

1.2 Objectives and Scope

The objectives of this research were to:

1. Establish the relative rates of nitrification for domestic wastewater treatment within an acclimated RBC fixed film system as a function of pH.
2. Observe and characterize the relative changes in the RBC biofilm as a function of pH.
3. Evaluate the efficacy of chemical addition to improve nitrification within an RBC fixed film system through the maintenance of an optimum pH.
4. Evaluate alternative alkaline chemicals for pH controlled nitrification for the RBC.
5. Develop design criteria, as appropriate, for pH controlled nitrification for the RBC.

Pilot scale 0.5 meter diameter RBC systems were used in this research to nitrify high rate trickling filter effluent from a university campus. This secondary effluent was typical of that wastewater normally associated with small communities, military installations, and other types of institutions. Such wastewaters are subjected to relatively large fluctuations in characteristics. The slow growing autotrophic nitrifying bacteria within nitrifying wastewater treatment systems do not respond rapidly either to short term or long term fluctuations in wastewater characteristics. These systems never are in true equilibrium because they are responding constantly to changing

wastewater parameters and changing secondary biofilm characteristics, such as heterotrophic activity. However, after the initial period of biofilm development is complete, the response functions of such nitrifying systems can be expected to fluctuate within certain limits. These limits define a state of "dynamic equilibrium." Within the context of this research, dynamic equilibrium is defined as a period of relatively constant ammonia removal.

In order to minimize variations within the pilot scale RBC nitrifying systems, certain parameters were controlled. The hydraulic loading to the RBC systems and peripheral RBC disc velocity were maintained at commonly accepted design levels throughout all phases of the research. The DO level was always above limiting conditions and the temperature was controlled to minimize the effect of daily, weekly, and seasonal variations. The elements of major research phases were conducted simultaneously so that systems' responses to the identical pattern of fluctuating wastewater characteristics could be compared on a relative basis.

SECTION II

MATERIAL AND METHODS

2.1 Experimental Apparatus

2.1.1 PSU Wastewater Treatment Plant

The secondarily treated wastewater (high rate trickling filter effluent) used in this research was taken from The Pennsylvania State University (PSU) wastewater treatment plant (WWTP). Figure 2.1 describes the overall wastewater treatment scheme of the facility. The PSU WWTP which is designed to handle approximately 9,500 m^3/d has primary aeration, primary sedimentation, high rate trickling filtration, secondary aeration (nitrification), secondary sedimentation, and chlorination. The adjacent State College Borough WWTP is an activated sludge facility with a 11,000 m^3/d capacity which occasionally bypasses a small fraction of the State College Borough raw influent wastewater to the headworks of the PSU facility. The effluents of both wastewater treatment plants are combined prior to chlorination. Although the two plants operate independently, both are owned and operated by PSU.

2.1.2 Pilot RBC Systems

A portion of the trickling filter effluent, which was recycled back to the influent of the trickling filters, was used for all phases of this research. A detailed description of the pilot RBC wastewater treatment facility used for each research phase is found in the beginning of the sections that follow.

2.2 Analytical Procedures

Appendix A contains a detailed summary of the wastewater and microbial biofilm sampling and analytical procedures. Any deviations from these methodologies are discussed in the sections where they occur.

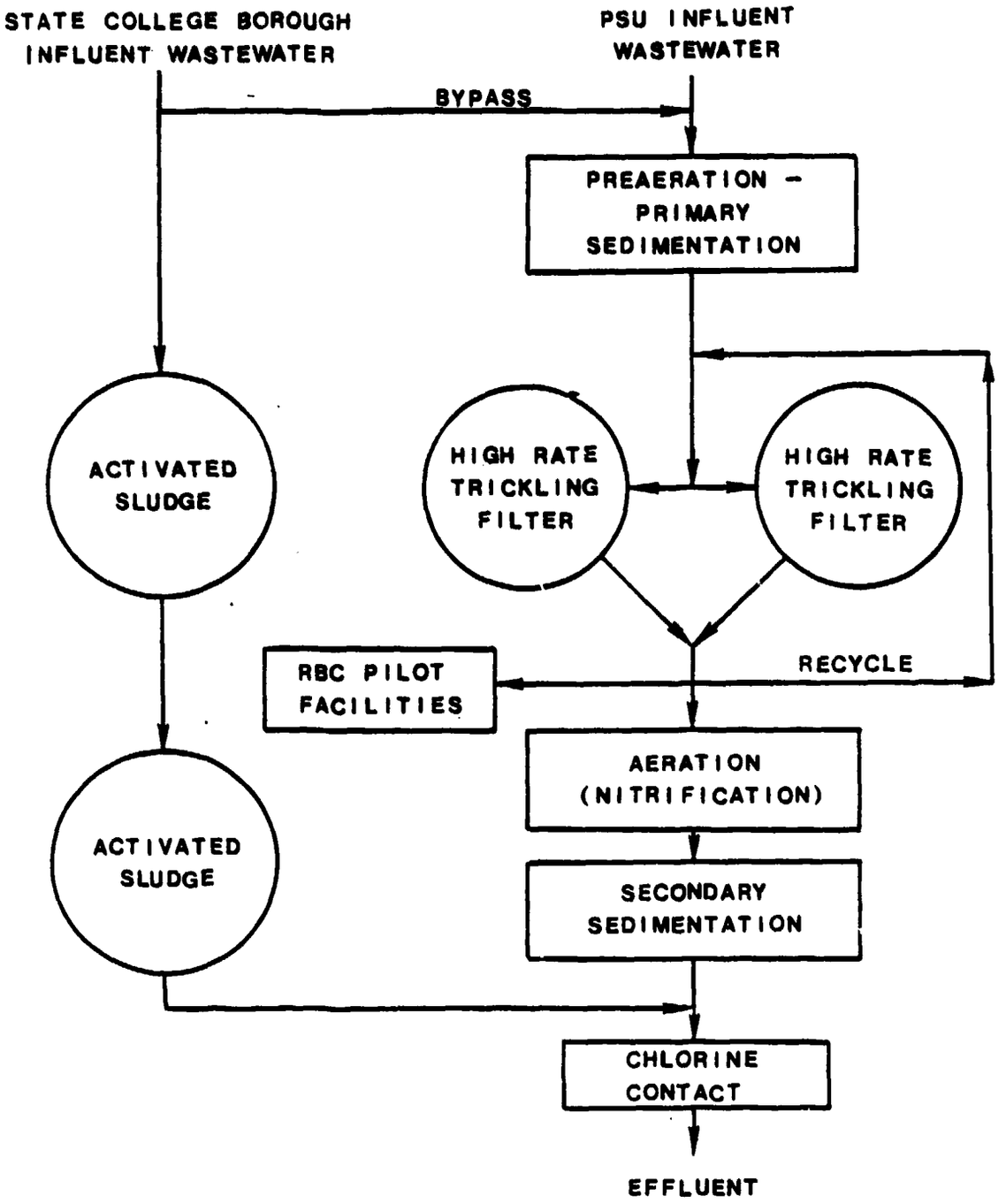


Figure 2.1 Schematic Diagram of The Pennsylvania State University (PSU) Wastewater Treatment Plant (WWTP)

SECTION III

NITRIFICATION OF HIGH RATE TRICKLING FILTER EFFLUENT WITH A PILOT SCALE 4-STAGE RBC

3.1 Introduction

The initial phase of the research was devoted to the successful establishment of a pilot scale 4-stage RBC WWTP capable of nitrifying high rate trickling filter effluent. The pilot RBC unit was operated for 50 days from 31 October until 19 December, 1979. Information was obtained regarding the characteristics of the RBC biofilm, the operational characteristics of nitrifying RBC systems, and the sampling and analytical procedures. Information and experience obtained in this initial research phase was beneficial in the RBC nitrification research described in the following sections.

3.2 Experimental Apparatus and Procedures

Figure 3.1 is a schematic diagram of the pilot 4-stage RBC system used to nitrify the domestic wastewater secondary effluent from the PSU WWTP high rate trickling filters. The operating characteristics of this pilot system are described in Table 3.1.

3.3 Experimental Findings

3.3.1 RBC Nitrification

The characteristics of the RBC wastewater influent are described in Table 3.2. It is obvious that the soluble carbonaceous BOD (CBOD) was sufficiently removed by the PSU WWTP's high rate trickling filters to allow nitrification to commence within the trickling filters and proceed within the pilot RBC. According to Antoine (7) soluble CBOD

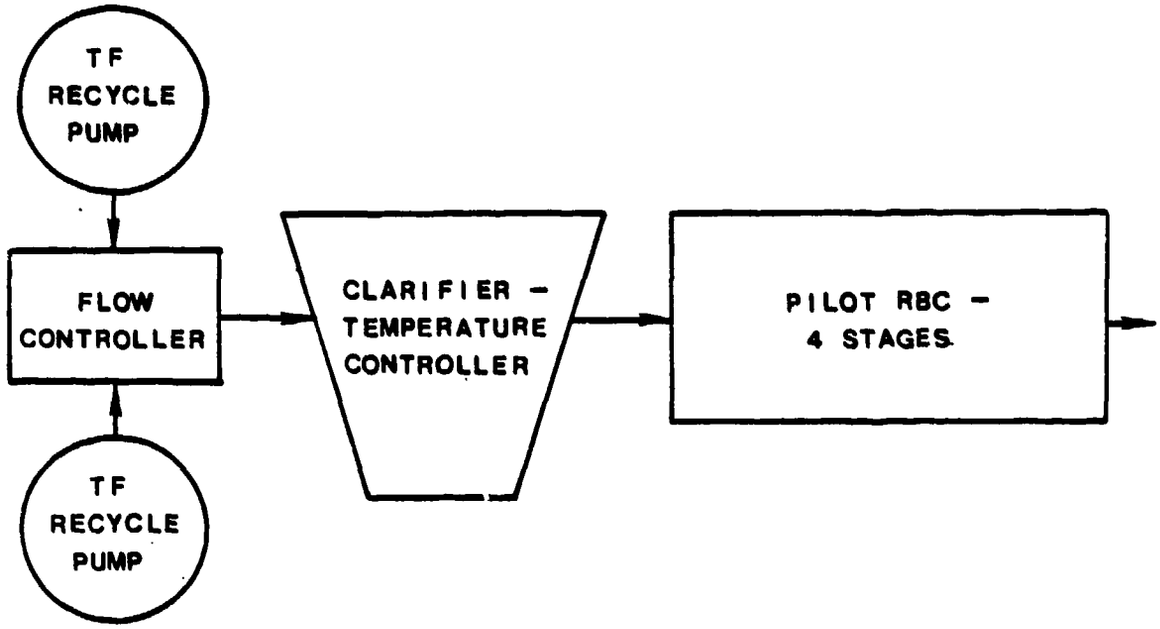


Figure 3.1 Schematic Diagram of the Pilot 4-Stage RBC Nitrification System

Table 3.1 Pilot 4-Stage Nitrifying RBC Operating Characteristics

Secondary Clarifier		
Surface Settling Rate (@1.7 m^3/d)	-	6.6 $m^3/d \cdot m^2$
Detention Time (@1.7 m^3/d)	-	2.7 hr
RBC		
Number of Stages	-	4
Discs per Stage	-	9
Disc Diameter	-	0.5 m
Disc Area - Total	-	21 m^2
Rotational Speed	-	13 rpm
Peripheral Speed	-	0.34 m/sec
Hydraulic Loading[a]	-	76 $l/m^2 \cdot d$

[a]The hydraulic loading of 76 $l/m^2 \cdot d$ (1.9 $gal/d \cdot ft^2$) was increased to 140 $l/m^2 \cdot d$ from Day 21 to Day 29 to provide additional substrate during the PSU Thanksgiving break.

should be reduced to between 15 and 20 mg/l before nitrification can proceed within a RBC. The mean CBOD entering the RBC pilot unit was 6.0 mg/l during the operational period. A breakdown of the BOD components remaining within the wastewater after secondary clarification and entering the pilot unit is presented in Table 3.3. Nitrification accounted for nearly half of the 5 day BOD whereas soluble CBOD accounted for approximately 30 percent. The remainder of the BOD was associated with particulate matter.

During this phase of the research the level of DO was greater than 5.0 mg/l in all the RBC stages and the DO increased by nearly 1 mg/l in

Table 3.2 Pilot 4-Stage Nitrifying RBC Influent Wastewater Characteristics[a]

Parameter	Mean	Std. Dev.
CBOD, mg/l	6.0(16)[b]	2.3
SS, mg/l	18 (21)	8
NH_3-N, mg/l	12.3(19)	4.4
$(NO_2 + NO_3)$-N, mg/l	3.4(19)	2.4
TKN-N, mg/l	18.3(18)	5.9
Alk., $\frac{mg\ CaCO_3}{l}$	275 (19)	37
pH	7.7(7)	-
Temperature, ^oC	20.2(39)	1.5

[a]Based upon samples taken of the RBC influent during the period 31 October to 19 December 1979.

[b]Number in parentheses is the number of samples applied to statistical determinations.

Table 3.3 BOD Components Entering the Pilot 4-Stage Nitrifying RBC

Component	BOD,[a] mg/l Mean	Std. Dev.	Percent of Total
Total - uninhibited	21.5(16)[b]	6.3	100
Total - inhibited	11.9(16)	3.9	55
Soluble - uninhibited	7.6(16)	2.1	35
Soluble - inhibited	6.0(16)	2.3	30

[a]BOD concentrations are 5 day values.

[b]Number in parenthesis is the number of samples applied to statistical determinations.

each successive stage. The mean RBC influent temperature was 20.2°C and decreased by approximately 2.5°C across the four RBC stages. The mean temperatures were 19.2, 18.6, 18.1, and 17.7°C for stages 1, 2, 3, and 4, respectively. The mean pH of RBC stage 1 was 7.6; however, the pH increased in stages 2 and 3 to pH 7.7 and to pH 7.9 in stage 4. The elevation of pH was due to the stripping of CO_2 from the wastewater.

The nitrification performance of the 4-stage RBC system is illustrated in Figure 3.2 Nitrification within the system commenced following an initial lag period of approximately 8 days. Between Day 8 and 13 the relatively slow growing ammonia-oxidizing bacteria developed a bacterial population sufficient to oxidize the influent ammonia. The nitrite-nitrogen concentration in the RBC effluent increased to 13.5 mg/l as is seen in the peaks for the nitrite-nitrogen curves in Figure 3.2 on Day 13. The resulting increase in the nitrite concentrations caused a subsequent increase in the nitrite-oxidizing bacterial population. By Day 17 the populations of both ammonia-oxidizing and nitrite-oxidizing bacteria were sufficient to completely oxidize most of the ammonia within the first two stages.

An obvious period of low ammonia influent and reduced wastewater strength occurred from Day 18 to Day 31. This transient condition related directly to the PSU Thanksgiving Day break of 16 to 26 November. During this period, the strength of the PSU wastewater declined and the nitrification capacity of the PSU WWTP high rate trickling filters increased thereby resulting in a dramatic drop in the RBC influent ammonia-nitrogen concentration. By Day 33 the influent wastewater strength as well as the operation of the pilot unit returned to near

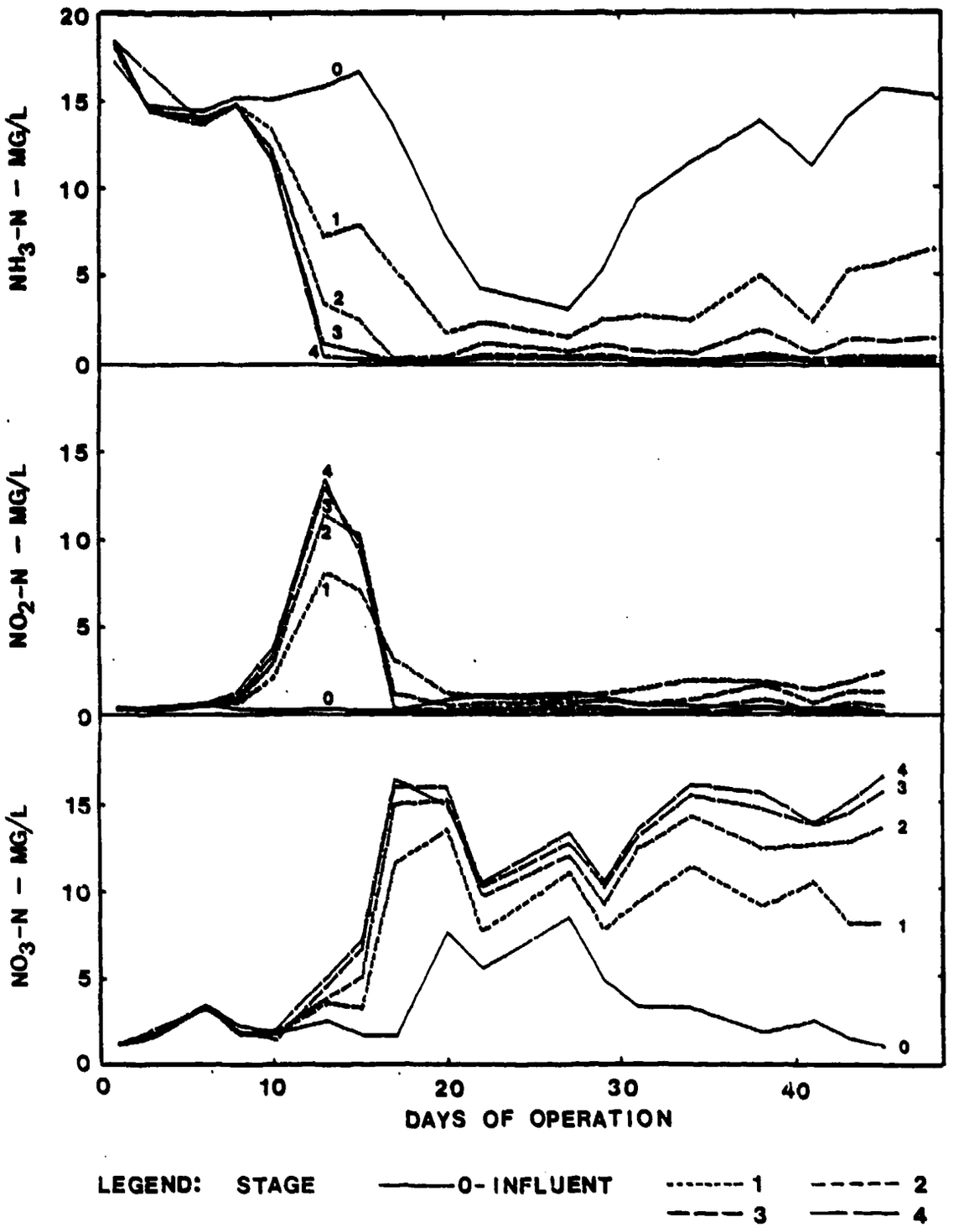


Figure 3.2 Ammonia-, Nitrite-, and Nitrate-Nitrogen Forms Within the 4-Stage Nitrifying RBC

normal and continued at this level for the remainder of this part of the study. Based upon the data presented in Figure 3.2 it is apparent that the major fraction of nitrification activity occurred within the first two stages of the RBC unit with more than half having occurred in the first stage. The third and fourth stages essentially acted to nitrify any remaining ammonia.

The wastewater data for the pilot RBC system were based upon timed grab samples taken in the morning only. The sampling started between 7 A.M. and 9 A.M. and took two hours to complete. In order to examine if the wastewater characteristics entering the pilot RBC system were significantly varying with time, individual hourly samples were taken of the PSU WWTP influent, the 4-stage RBC pilot plant influent, and RBC Stage 1 from 9 A.M. on Day 48 until 8 A.M. on Day 49. The ammonia-nitrogen concentrations for this period are shown in Figure 3.3 and Figure 3.4. The PSU WWTP influent demonstrated a rather dramatic diurnal variation in ammonia-nitrogen concentration. The level of ammonia-nitrogen entering the PSU WWTP varied from 4.1 mg/l to 30.6 mg/l. In addition, approximately 1 mg/l of completely oxidized nitrogen entered the PSU WWTP between 3 A.M. and 5 A.M. The appearance of these oxidized nitrogen species in the early morning, when the concentrations of influent ammonia-nitrogen and organics were low, clearly established the existence of viable populations of ammonia-oxidizing and nitrite-oxidizing bacteria within the sewer system. The rapid and short lived appearance of nitrite and nitrate raises the distinct possibility of bacterial inhibition associated with the increased organics in the higher strength wastewater.

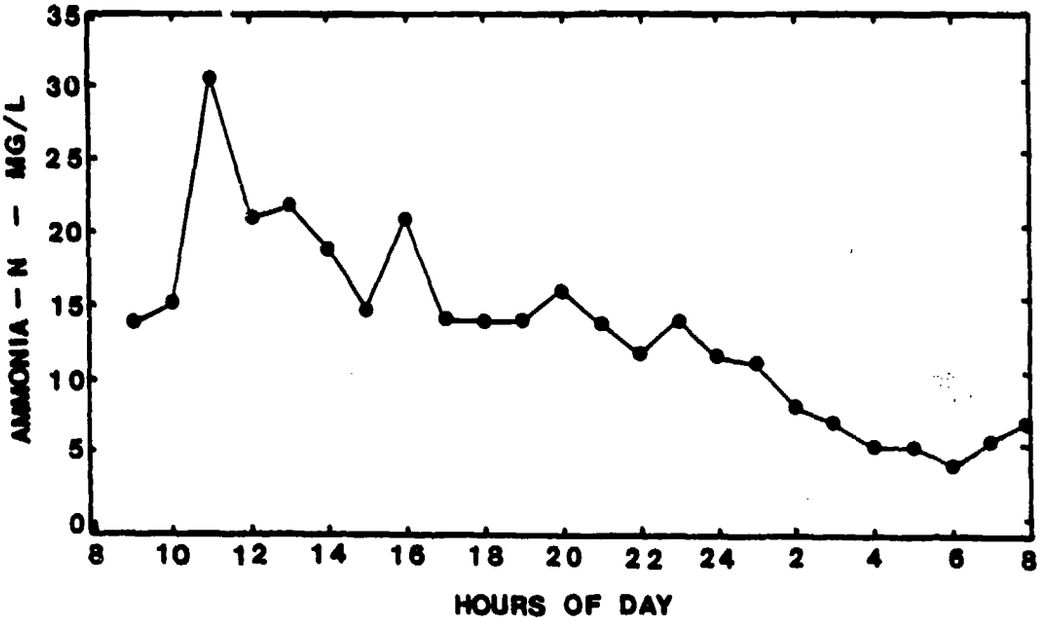


Figure 3.3 Diurnal Variation of Ammonia-Nitrogen in the PSU WWTP Influent, 17-18 December, 1979

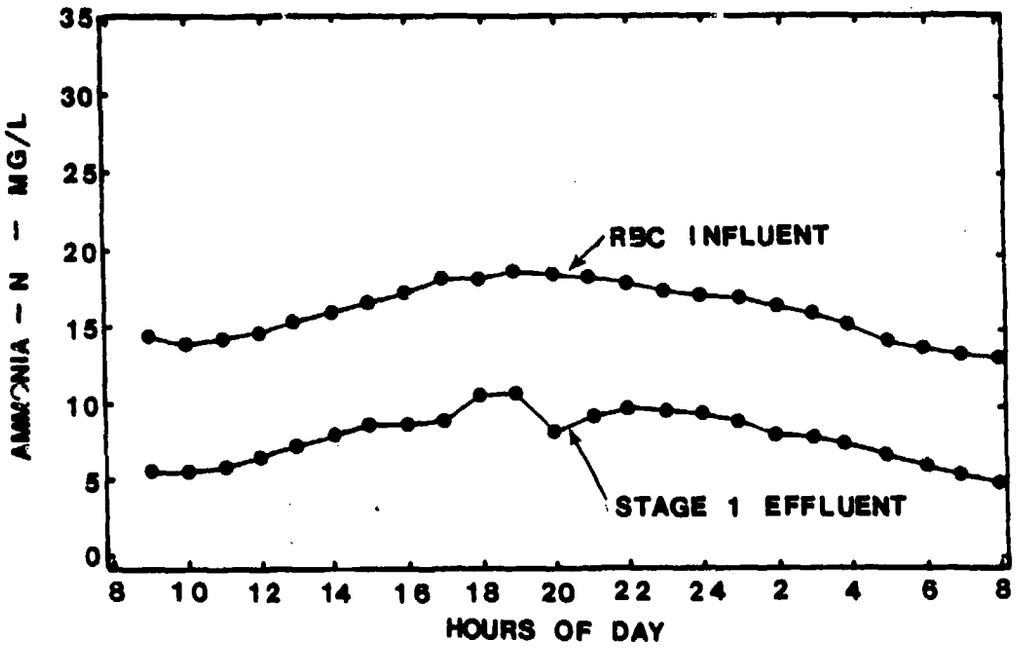


Figure 3.4 Diurnal Variation of RBC Influent (Top) and Effluent Ammonia-Nitrogen (Bottom) for the First Stage of the 4-Stage Nitrifying RBC, 17-18 December, 1979

Figure 3.4 clearly demonstrated that the influent wastewater to the pilot system, as well as wastewater within the RBC stages, experienced significant diurnal variations in wastewater parameters and that grab sampling during the morning hours alone may not have produced the most representative results. Combining these data with three other sets of grab and composite sampling data demonstrated that the RBC influent ammonia-nitrogen levels were 23 percent higher for the composited samples. As a result of these findings, all subsequent sampling utilized dual timed grab composited samples wherein each set of daily samples was composed of two sets of timed grab samples (see Appendix A). The first set was taken in the evening when the RBC influent wastewater concentrations were maximum and composited with samples taken early the next morning when wastewater concentrations were minimum. The net effect of this procedure was to produce wastewater parameter concentrations representative of means obtained by composite sampling.

3.3.2 Biofilm Development and Microbial Enumerations. The initial biofilm development was very uniform in texture, bronze to brown in color, relatively thin, and decreasing in thickness with succeeding stages. Biofilm sloughing occurred in stage 1 on Day 21, in stage 2 on Day 25, and in stages 3 and 4 on Day 38. This initial sloughing appeared to be associated with the reduced strength of the influent wastewater during the PSU Thanksgiving recess. Although the biofilm was reestablished, it never returned to its original highly uniform texture. The biofilm became more irregular and darker with age and increasing weight. Snails which infested the trickling filter first were observed within the troughs on Day 30 and their population increased with time. Although they were present on all the stage walls,

they never took up residence on the discs. The biofilm development for all four stages is described in Figure 3.5. The pattern of disc development clearly shows the heavier growth expected in the initial stages which was associated with the higher levels of CBOD and ammonia. Even after seven weeks of operation, these curves indicate that not all the biofilms had reached an equilibrium mass concentration wherein biofilm production was matched by biofilm loss due to hydraulic shear. The maximum rate of ammonia oxidation which occurred in RBC stage 1 on Day 13 corresponded to a stage active biofilm level of approximately 0.70 mg/cm^2. After achieving this level of biofilm density, the ammonia oxidation performance of RBC 1 did not increase, yet the biofilm solids continued to accumulate. After seven weeks of operation, the biofilm on stage 1 was approximately 4 mg/cm^2. The biofilm accumulated to a mean thickness of approximately a millimeter on stage 1 and to lesser depths on successive stages. The percent volatile matter within each of the biofilms was 84, 84, 81, and 85 percent for stages 1, 2, 3, and 4, respectively.

In this initial test phase, the biofilm solids were determined from samples removed from preplaced mylar squares located near the periphery of the plexiglass disc in each of the RBC stages (see Appendix A). These peripheral test areas were biased heavily toward the outer portions of the disc surface. In order to establish if the biofilm distribution on the plexiglass disc was a function of distance from the center of the disc, rectangular mylar sections also had been preplaced radially outward from the RBC shaft within each stage. The sampling areas of these rectangular sections were biased toward the center. The results of this test are presented in Table 3.4. More biofilm was concentrated near the

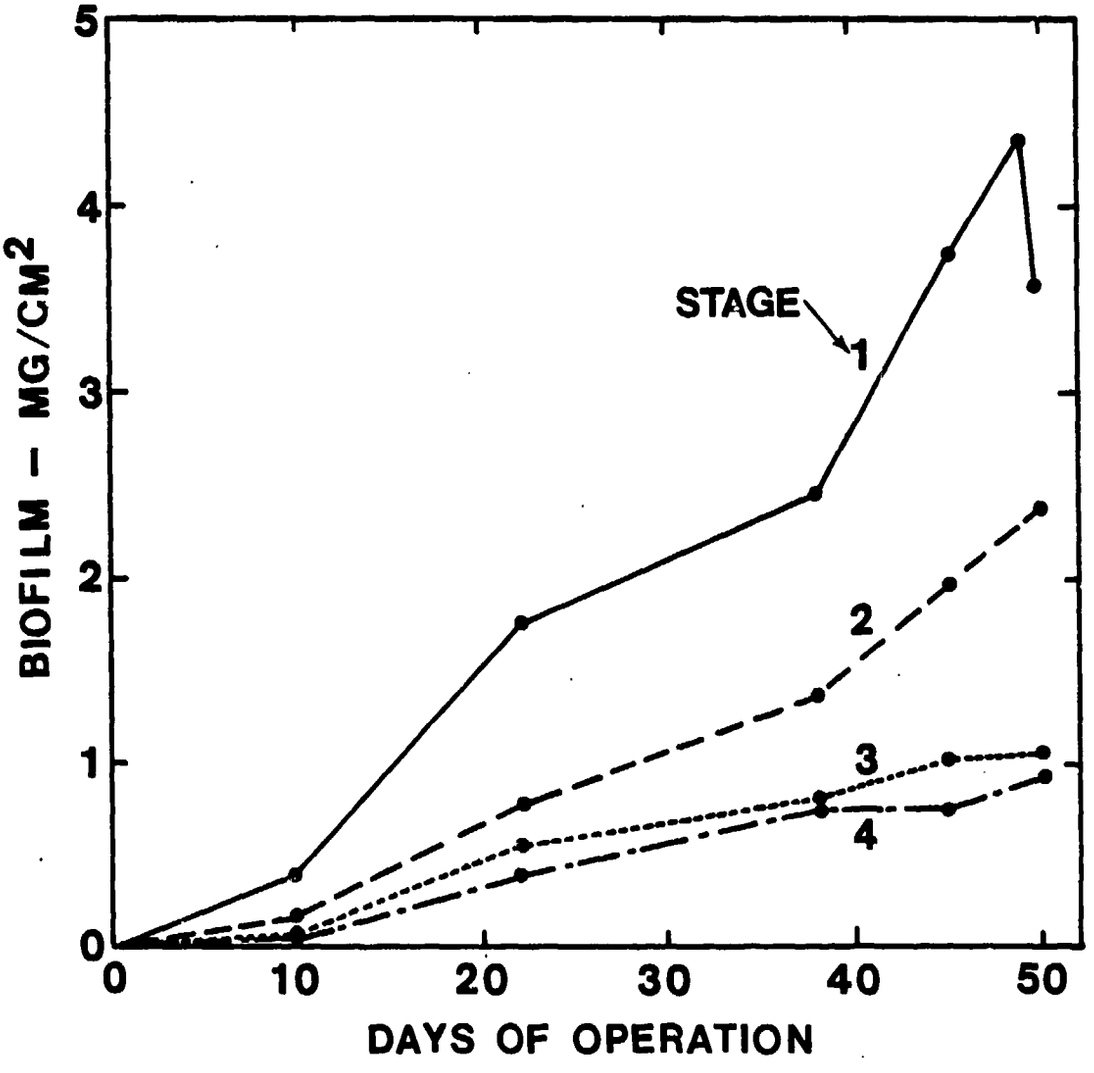


Figure 3.5 Biofilm Development on the Discs of a 4-Stage Nitrifying RBC

Table 3.4 Radial Comparison of Biofilm Solids on the Pilot 4-Stage Nitrifying RBC[a]

RBC Stage	Peripheral Solids mg/cm^2	Peripheral Solids cm^2	Radial Solids mg/cm^2	Radial Solids cm^2
1	3.59	18[b]	1.98	194[c]
2	2.38	18	1.11	258
3	1.07	18	0.54	258
4	0.91	18	0.64	258

[a]Sampling date was Day 50.

[b]Peripheral solids were collected on 9 cm^2 mylar squares with the centers mounted 4.5 cm from the edge of the plexiglass disc.

[c]Radial solids were collected on 64.5 cm^2 mylar rectangles (3 cm x 21.5 cm) mounted flush with the edge of the plexiglass disc and extending toward the center.

outer portions of the disc than toward the center. Obviously the results indicate that a radial bias in the mass of biofilm did exist. This condition is attributed to increased turbulence nearer the edge allowing increased DO and substrate diffusion.

The population data for ammonia-oxidizing and nitrite-oxidizing bacteria within the four stages on a unit disc area and unit weight basis are illustrated in Figures 3.6 and 3.7, respectively. The data within each figure are based upon the geometric means of two sets of samples taken during the last two weeks of operation (Days 45 and 50). In general, it can be seen that the number of ammonia-oxidizers was greater than the nitrite-oxidizers by approximately an order of magnitude. The populations of both groups of organisms decreased across the

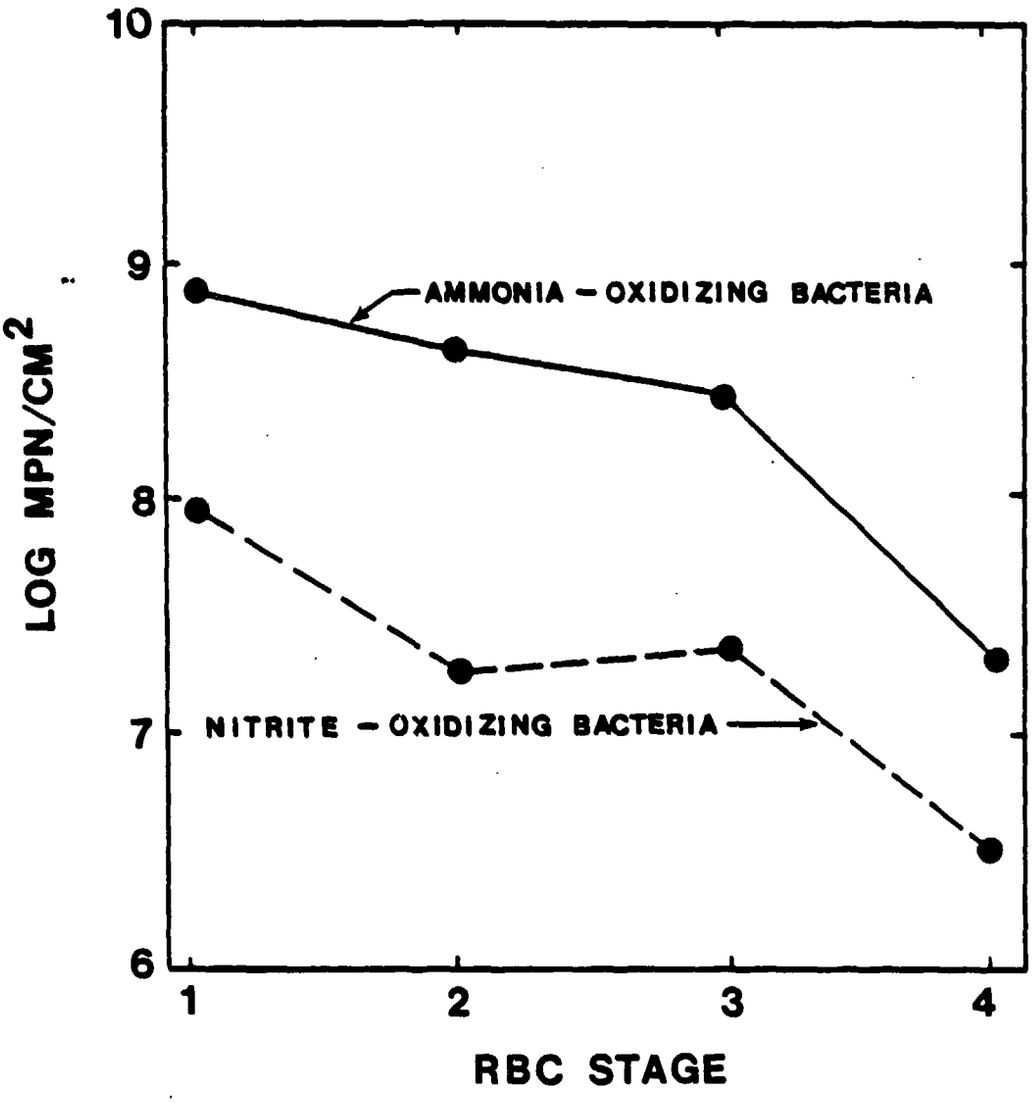


Figure 3.6 Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria per Unit Disc Area of a 4-Stage Nitrifying RBC

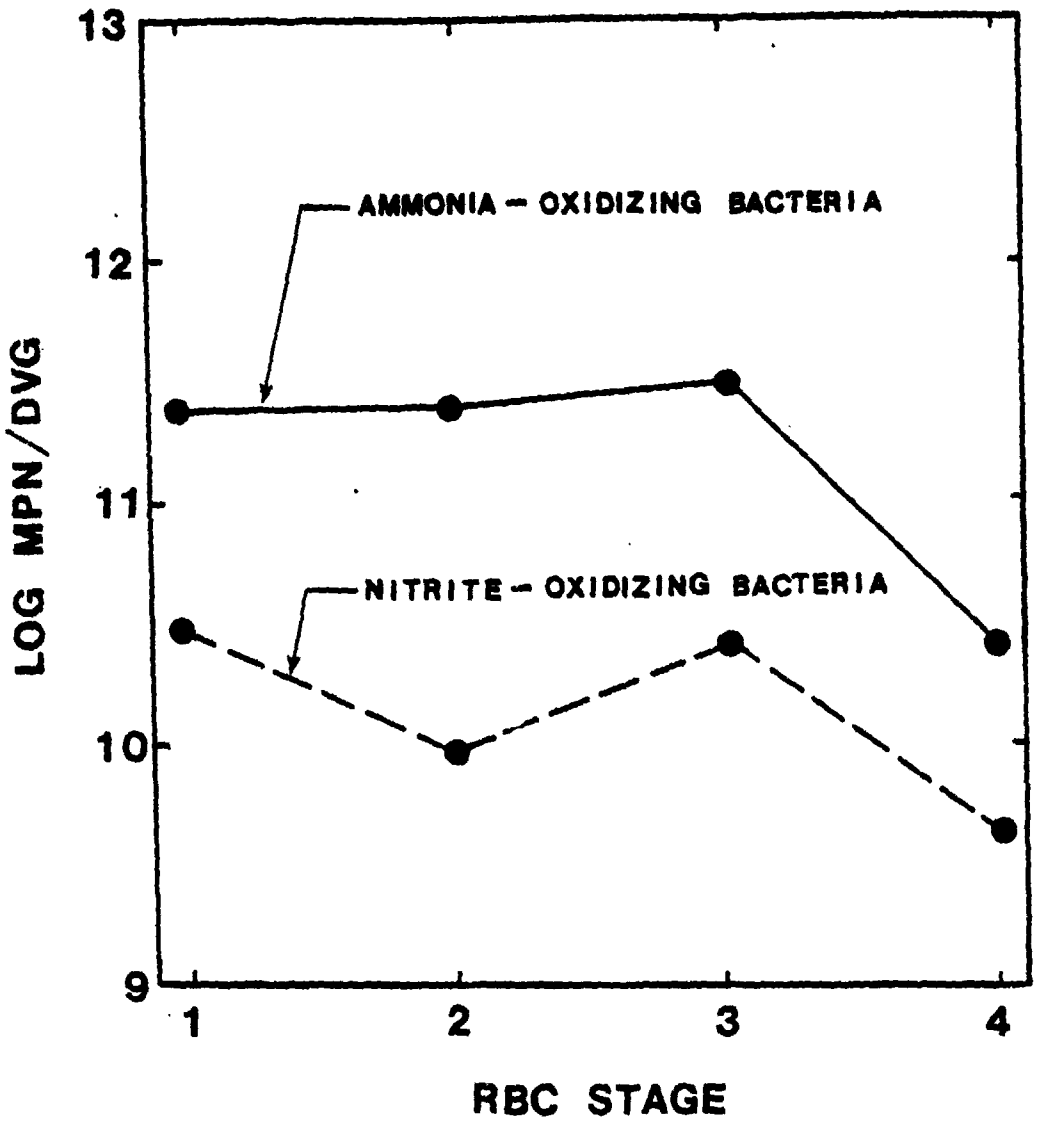


Figure 3.7 Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria per Unit Weight of Dry Volatile Biofilm of a 4-Stage Nitrifying RBC

four stages of the RBC by approximately an order of magnitude. The greatest part of this drop occurred in stage 4 where comparatively little nitrification activity occurred. The mean ratio of ammonia-oxidizers to nitrite-oxidizers for all four stages was 14 to 1. The activity levels for each stage based upon the ammonia-oxidizer and nitrite-oxidizer population data presented in Figure 3.6 are presented in Table 3.5.

Table 3.5 Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria Activity Levels[a] for Pilot 4-Stage Nitrifying RBC.

RBC Stage	Ammonia-Oxidizers NH_3-N Removed		Nitrite-Oxidizers NO_2-N Removed	
	mg/l	pmole cell-hr	mg/l	pmole cell-hr
1	9.1	0.0011	7.2	0.0071
2	3.7	0.00079	3.9	0.021
3	1.0	0.00031	1.7	0.0071
4	0.2	0.00093	0.6	0.010

[a]Calculations are based upon mean chemical data from Day 38 to 49, 30 minute detention time, 5.3 m^2 disc area, and microbial populations in Figure 3.6.

SECTION IV

RBC NITRIFICATION OF HIGH RATE TRICKLING FILTER EFFLUENT

pH 6.3 - pH 7.5

4.1 Introduction

This research phase was devoted to the evaluation of the relative rates of nitrification of domestic wastewater effluent from a high rate trickling filter within RBC systems maintained at normal and below normal pH levels. Based upon the work described in Section III, it was clear that by observing the performance of the first RBC stage the effect of pH on the nitrification process could be assessed adequately. Therefore, four single stage nitrifying RBC systems were operated which simultaneously treated the same influent wastewater thereby experiencing the same cyclic variations in wastewater characteristics. The pH and alkalinity levels were adjusted downward artificially and maintained at three different levels within three of the RBC systems. The fourth RBC system served as the control. The nominal hydraulic loading to each RBC system was 81 $l/m^2 \cdot d$ (2 $gal/d \cdot ft^2$). This research phase originally was scheduled for six weeks; however, it was lengthened to ten weeks in order to obtain additional operating data. The RBC systems were operated from 20 January, 1980 until 28 March, 1980.

4.2 Experimental Apparatus and Procedures

The effluent from the PSU WWTP high rate trickling filters, Figure 2.1, was passed through a flow controller, a plexiglass secondary clarifier, and a flow divider, which split the wastewater flow equally into four separate channels, and then into the four RBC systems. For purposes

of this study, each RBC consisted of one of the stages of a 4-stage 0.5 meter diameter pilot RBC so the RBC systems operated in parallel. This modification of the pilot RBC made it possible to observe simultaneously the relative rates of nitrification under varying pH and alkalinity levels while the other influent wastewater characteristics and operational conditions were common to all four RBC systems. Low pH-low alkalinity environments were created by adding various concentrations of sulfuric acid into three of the four wastewater flow channels of the flow divider just prior to the flow entering into each of the completely mixed RBC systems. The fourth RBC, which served as the control, treated the unaltered wastewater. Ammonium chloride was fed continuously into the pilot plant facilities during the PSU spring break to prevent a severe ammonia depletion during the break. A schematic diagram is shown in Figure 4.1 and the operational characteristics of the RBC systems are presented in Table 4.1. Routine sampling of the four RBC influents and four RBC effluents was performed five days per week for the duration of the study. All other sampling and analytical procedures are described in Appendix A.

4.3 Experimental Findings

4.3.1 Relative Rates of Nitrification

Data on the amounts of ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen entering and leaving the RBC systems throughout the entire study period are presented graphically in Figure 4.2. The data on the relative amounts of ammonia removed by each RBC system, as a function of pH and time, are presented in Figure 4.3. The initial rates of nitrification which developed during the first month of operation clearly

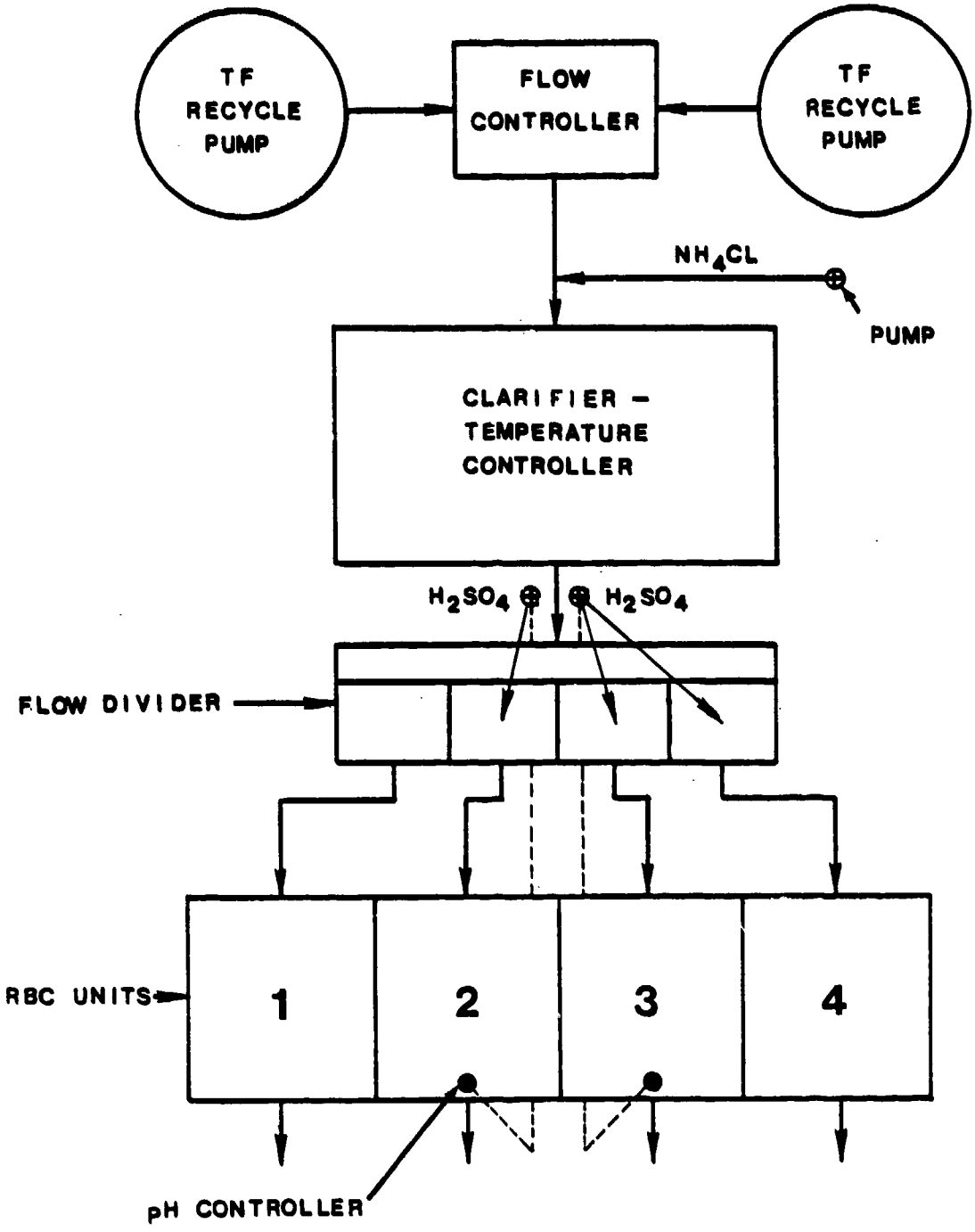


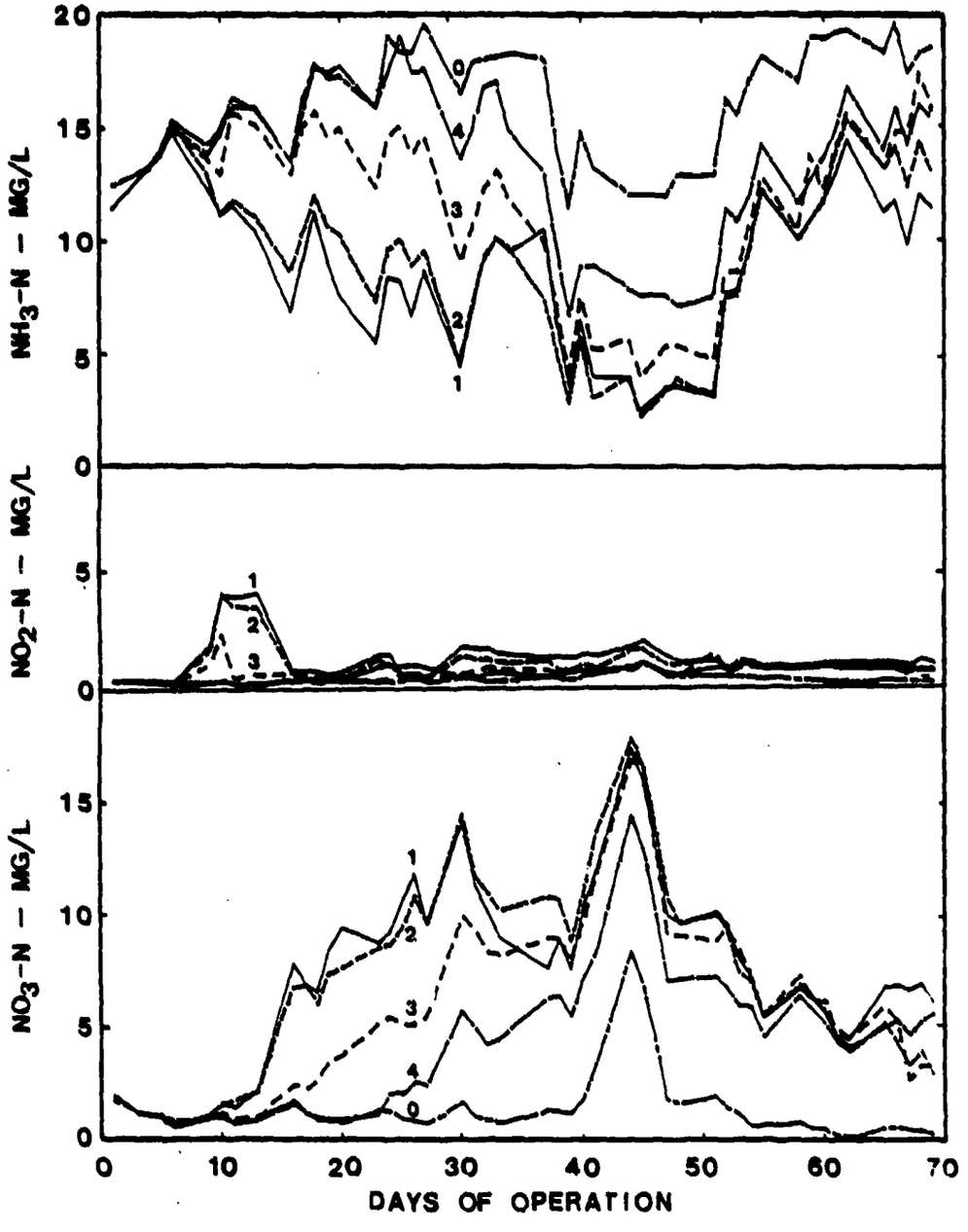
Figure 4.1 Schematic Diagram of the Pilot RBC Units for the Low pH-Nitrification Study

Table 4.1 Pilot Single-Stage Nitrifying RBC Operating Characteristics

Secondary Clarifier		
Surface Settling Rate (@ 6.8 m^3/d)	-	5.9 $m^3/d \cdot m^2$
Detention Time (@ 6.8 m^3/d)	-	2.1 hr
RBC		
Number of RBCs	-	4
Stages per RBC	-	1
Discs per Stage	-	9
Disc Diameter	-	0.5 m
Disc Area - Total	-	5.3 m^2
Rotational Speed	-	13 rpm
Peripheral Speed	-	0.34 m/sec
Hydraulic Loading[a]	-	81 $l/m^2 \cdot d$

[a]The hydraulic loading for all four RBC units was nominally 81 $l/m^2 \cdot d$ (2 $gal/d \cdot ft^2$), the exact hydraulic loading for each RBC is found in Table 4.2. The hydraulic loading calculation is based upon the assumption that each RBC is the first stage of a 4-stage RBC.

were related to pH. The higher initial rates observed were associated with higher pH levels. The maximum rates of nitrification for the pH 7.5 and 7.1 RBC systems occurred after approximately a month of operation. These initial rates of 3.3 to 3.9 g H_3-N/$d \cdot m^2$ were much higher than have been reported for nearly identical systems operating under similar conditions (Miller (71), Borchardt (16), and Antoine (7)). Both of these systems then experienced a decline in performance and never returned to these relatively high maximum rates observed initially. The



LEGEND: RBC —·— 0-INFLUENT ——— 1-pH 7.5 ------- 2-pH 7.1
— — — 3-pH 6.5 — — 4-pH 6.3/6.7

Figure 4.2 Influent and Effluent Nitrogen Forms for the RBC Units of the Low pH-Nitrification Study

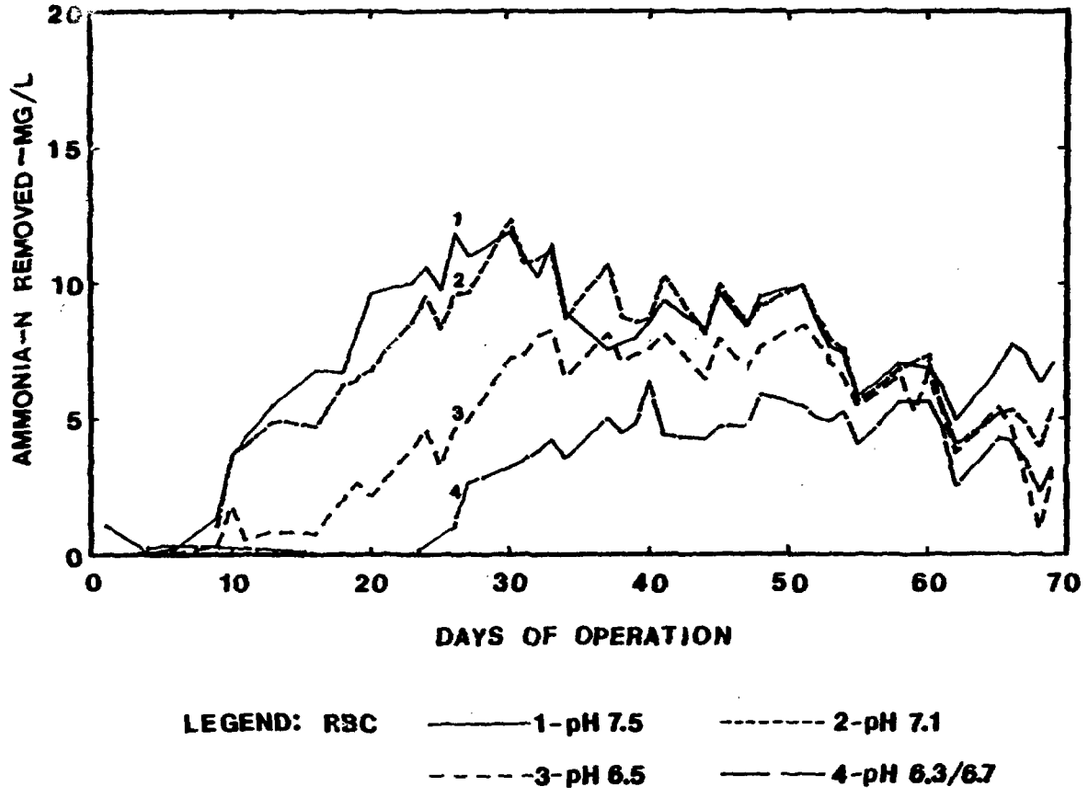


Figure 4.3 Relative Ammonia Removals for the RBC Units of the Low pH-Nitrification Study

nitrification performance of the RBC system at pH 6.5 clearly developed at a much slower initial rate and never achieved the same nitrification rates as observed for the two higher pH RBC systems. The RBC system which was maintained at pH 6.3 was not successful in establishing nitrification during the first 25 days of operation. On Day 5, the pH 6.3 RBC system experienced a short-term pH excursion down to pH 2.9 for approximately one-half hour. Similarly, on Day 10, another pH excursion down to pH 2.8 was experienced for an estimated one hour. These two pH excursions may have had an adverse initial effect on the development of the nitrification process within this low pH system. On Day 27, the pH of this RBC unit was adjusted upward to pH 6.7 in an attempt to obtain additional information regarding the nitrification rate between pH 6.5 and pH 7.1; this RBC unit is referred to hereafter as the pH 6.3/6.7 RBC. The rate of nitrification for this RBC did improve; however, the level of performance remained significantly lower than the pH 6.5 RBC for the next 30 days. The performance of the pH 6.3/6.7 RBC did not surpass the performance of the pH 6.5 RBC until Day 67 which was 40 days after the pH adjustment.

Based upon nitrification performance, a period of relative dynamic equilibrium was established approximately on Day 37 for the pH 7.5, 7.1, and 6.5 RBC units. A detailed comparison of the RBC units' influent and effluent wastewater characteristics from Day 37 until the end of the study on Day 69 is presented in Tables 4.2 and 4.3, respectively. Data on the relative amounts of ammonia-nitrogen removed by the RBCs are presented in Table 4.4. The rate of nitrification for the pH 7.1 RBC system was 96 percent of that observed at pH 7.5 and the rate for the pH 6.5 RBC was 80 percent of the rate for the pH 7.5 RBC. Because of the

Table 4.2 RBC Influent Wastewater Characteristics for the Low pH-Nitrification Study[a]

Parameter	RBC 1 (pH 7.5) Mean	Std. Dev.	RBC 2 (pH 7.1) Mean	Std. Dev.	RBC 3 (pH 6.5) Mean	Std. Dev.	RBC 4 (pH 6.3/6.7) Mean	Std. Dev.
Alk($CaCO_3$), mg/l	228 (19)[b]	30	196 (18)	27	110 (21)	29	106 (17)	51
CBOD, mg/l	6.0 (13)	3.0	5.3 (16)	2.6	5.7 (14)	2.5	5.4 (13)	2.1
SS, mg/l	17 (23)	3	18 (22)	3	19 (24)	4	17 (23)	3
VSS, %	85 (23)	3	86 (22)	4	87 (23)	3	87 (23)	3
TKN-N, mg/l	17.6 (23)	3.2	17.7 (23)	3.2	17.9 (24)	3.2	17.6 (23)	3.3
NH_3-N, mg/l	15.9 (23)	2.8	15.9 (23)	2.8	16.1 (24)	2.8	16.1 (23)	2.8
Org-N, mg/l	1.7 (23)	1.0	1.8 (23)	0.9	1.8 (24)	1.0	1.6 (23)	1.0
(NO_2 + NO_3)-N, mg/l	1.9 (23)	2.3	1.9 (23)	2.2	1.8 (24)	2.2	2.0 (23)	2.5
NO_2-N, mg/l	0.3 (23)	0.3	0.3 (23)	0.3	0.3 (24)	0.2	0.3 (23)	0.2
NO_3-N, mg/l	1.6 (23)	2.0	1.6 (23)	2.0	1.5 (24)	2.0	1.7 (23)	2.2
Flow, $l/m^2 \cdot d$	82 (33)	7	79 (33)	5	82 (33)	7	81 (33)	6

[a]Based upon data from Day 37 to Day 69.

[b]Number in parenthesis is the number of samples applied to statistical determinations.

Table 4.3 RBC Effluent Wastewater Characteristics for the Low pH-Nitrification Study[a]

Parameter	RBC 1 (pH 7.5) Mean	Std. Dev.	RBC 2 (pH 7.1) Mean	Std. Dev.	RBC 3 (pH 6.5) Mean	Std. Dev.	RBC 4 (pH 6.3/6.7) Mean	Std. Dev.
pH	7.5 (33)[b]	-	7.1 (33)	-	6.5 (33)	-	6.7 (33)	-
Alk($CaCO_3$), mg/l	173 (23)	39	127 (23)	33	59 (24)	12	85.9 (23)	19
CBOD, mg/l	4.3 (13)	1.3	3.7 (15)	1.2	3.9 (14)	1.2	4.1 (13)	1.2
SS, mg/l	15 (23)	3	15 (23)	3	17 (24)	4	16 (23)	3
VSS, %	87 (23)	3	86 (23)	3	88 (24)	2	87 (23)	2
TKN-N, mg/l	9.7 (23)	3.9	10.3 (23)	4.6	11.8 (24)	4.5	13.2 (23)	3.7
NH_3-N, mg/l	8.2 (23)	3.8	8.5 (23)	4.5	10.0 (24)	4.3	11.5 (23)	3.3
Org-N, mg/l	1.5 (23)	0.5	1.8 (23)	0.9	1.8 (23)	0.8	1.6 (23)	0.9
(NO_2-NO_3)-N, mg/l	9.6 (23)	3.5	9.4 (23)	4.0	8.5 (24)	3.7	7.0 (23)	2.8
NO_2-N, mg/l	1.1 (23)	0.3	0.9 (23)	0.3	0.7 (24)	0.3	0.7 (23)	0.2
NO_3-N, mg/l	8.5 (23)	3.3	8.5 (23)	3.8	7.8 (24)	3.6	6.3 (23)	2.7
SO_4, mg/l	26 (8)	4	74 (8)	12	173 (7)	37	142 (7)	31
DO, mg/l	5.5 (22)	0.8	5.5 (22)	0.7	6.3 (22)	0.7	7.1 (22)	0.7
Temp. °C	19.2 (33)	1.5	19.3 (33)	1.5	19.3 (33)	1.5	19.3 (33)	1.5

[a]Based upon data from Day 37 to Day 69.

[b]Number in parenthesis is the number of samples applied to statistical determinations.

relatively long period of time required for the pH 6.3/6.7 RBC to establish its nitrification process, data for this unit are not included in Table 4.4

Table 4.4 Relative Rates of Nitrification for RBC Systems Operating Under Low pH Conditions[a]

RBC pH	Ammonia-N Removed g NH_3-N/m^2·d	Percent of Maximum
7.5	2.5	100
7.1	2.4	96
6.5	2.0	80

[a]Based upon data from Day 37 to Day 69.

Nitrogen balances for the four RBC's are presented in Table 4.5. The slightly lower nitrogen recoveries for the two higher pH systems might reflect small nitrogen losses associated with denitrification within the heavier biofilms as well as minor losses due to ammonia stripping.

Table 4.5 RBC Nitrogen Balances for the Low pH-Nitrification Study[a]

RBC	pH	Total Nitrogen[b] - mg/l Influent	Effluent	Percent Recovery
1	7.5	19.5(23)[c]	19.3(23)	99
2	7.1	19.6(23)	19.7(23)	100
3	6.5	19.7(24)	20.3(24)	103
4	6.3/6.7	19.6(23)	20.2(23)	103

[a]Based upon data from Day 37 to Day 69

[b]Total nitrogen is total oxidized nitrogen plus total Kjeldahl nitrogen (TKN).

[c]Number in parenthesis is the number of samples utilized in the total nitrogen determinations.

Figure 4.4 presents the CBOD removal data for the four RBC systems as a function of time. The influent level of CBOD dropped just before and during the PSU term break which occurred from Day 35 to Day 51. The CBOD level increased again with the start of the spring term as the PSU student population suddenly increased and the trickling filters experienced a period of readjustment. The return of the student population and the subsequent increase in the influent CBOD level resulted in an increased heterotrophic activity on the RBC discs. Some of the slow growing nitrifying bacteria were displaced from the active layer of biofilm by heterotrophic bacteria and, therefore, the nitrification performance dropped in all four RBC systems. The recovery of the nitrification performance was related to pH just as it had been during the initial start-up period with the higher pH system responding more rapidly. It was during this regrowth and readjustment period, that the pH 6.3/6.7 RBC developed a level of nitrification greater than that of the pH 6.5 RBC.

4.3.2 Biofilm Development and Microbial Enumerations

The study was begun with no biofilm on the discs; however, all four RBCs developed biofilms which could be sensed by touch within 48 hours. A noticeable bronze color developed after five days of operation. By the tenth day, all RBC systems had developed thin and highly uniform textured coatings which possessed a visually apparent gradation. The heaviest biofilm appeared in the pH 7.5 RBC and the lightest growth of biofilm was on the pH 6.3/6.7 RBC. All four RBC units showed some degree of sloughing by Day 19 with the greatest sloughing occurring in the pH 6.3/6.7 RBC. The biofilm color changed from bronze to brown with age and increasing biomass. After the loss of the initial biofilm uniformity, the biofilm became increasingly patchy with time and

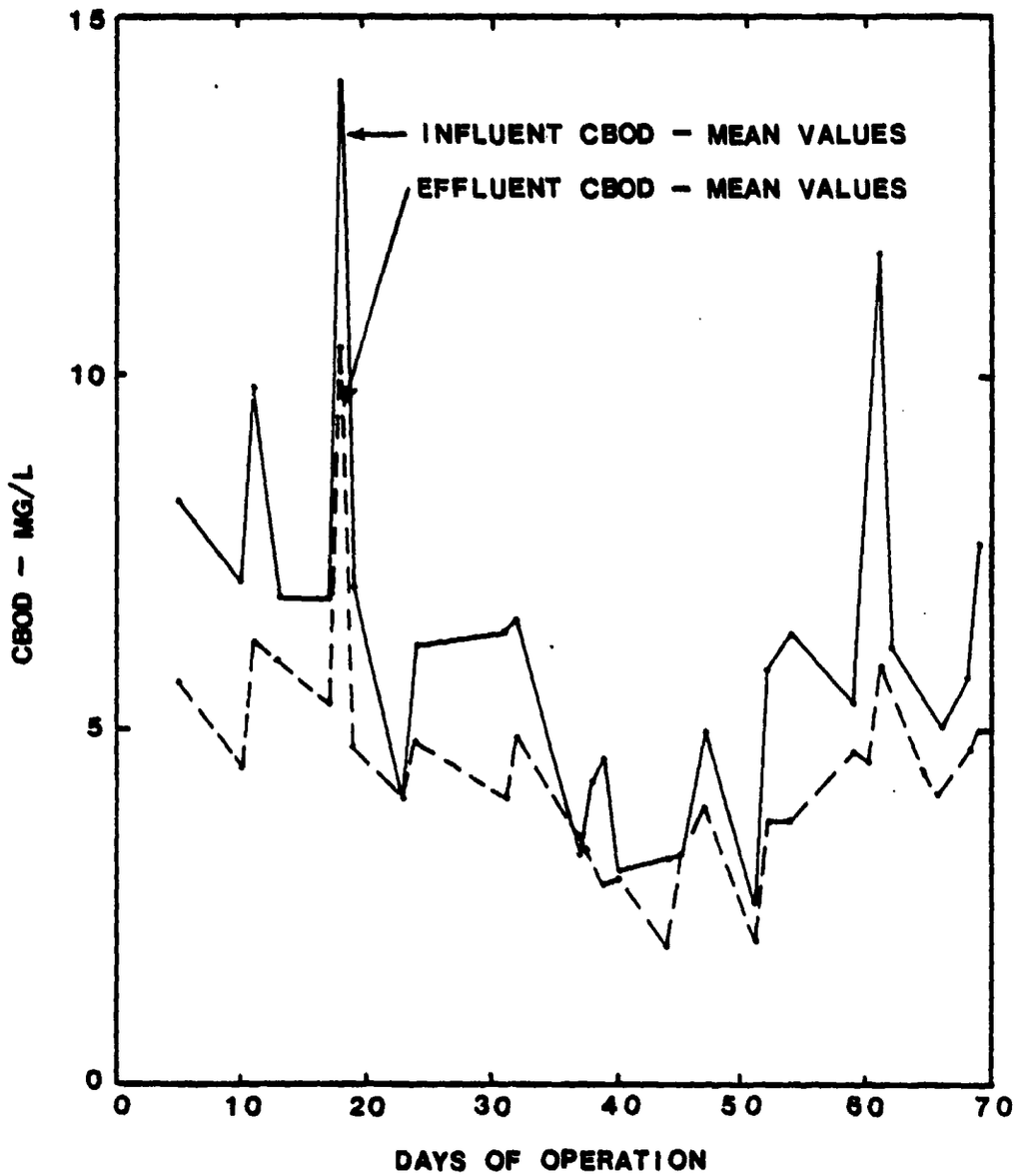
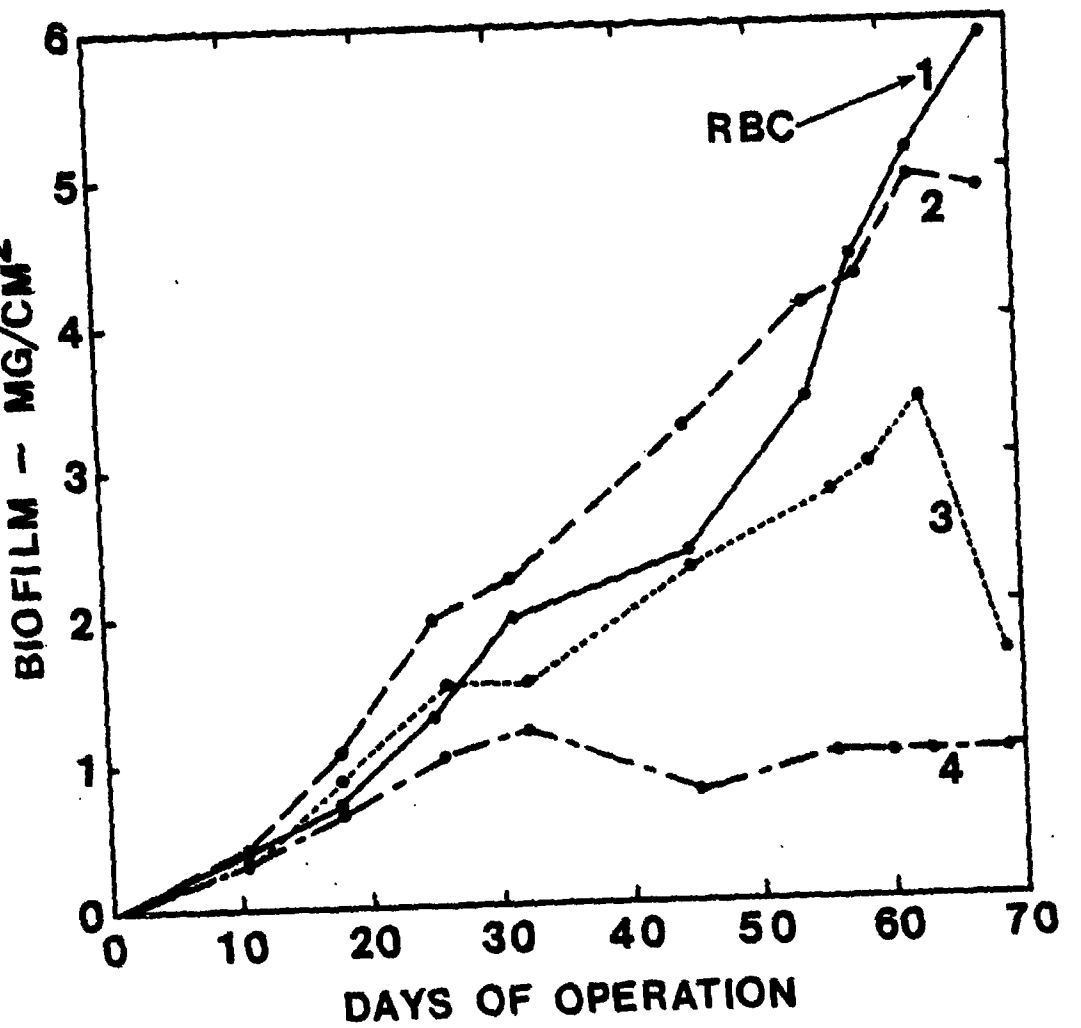


Figure 4.4 Influent and Effluent CBOD for the RBC Units of the Low pH-Nitrification Study

decreasing pH. At the conclusion of this study, the nonuniformity of the biofilm was quite evident visually (Figure B.1, Appendix B) and related directly to the relative nitrification rates recorded over the duration of the study. The patchy appearance was attributed mainly to biofilm loss resulting from hydraulic shear. However, biofilm sloughing down to the disc surface, as noted above, did occur. Snails coming from the trickling filters were noted first in all the RBC troughs on Day 24. They lived on the trough walls and did not inhabit the disc surface area. The numbers of snails observed were greater for the two higher pH systems.

Figure 4.5 presents quantitatively the RBC disc biofilm development data for all four RBC systems during the low pH-nitrification study. The RBC systems at pH 7.5 and 7.1 showed the best performance and had the most disc biofilm. The pH 6.5 RBC showed a lower level of performance and less biofilm. The RBC which was operated initially at pH 6.3 and later adjusted to pH 6.7 had the lowest performance level throughout most of the 69-day study and also developed the least amount of biofilm. The maximum ammonia-oxidation levels for the pH 7.5, 7.1, and 6.5 RBC's were achieved when the biofilm masses were approximately 2.0, 2.2, and 1.5 mg/cm^2 respectively. As demonstrated previously in Section 3.3.2, increases in disc biomass did not enhance the nitrification rates for any of these RBC systems. The pH 6.3/6.7 RBC added biofilm during the first three weeks of operation at a rate comparable to that of the pH 7.5 RBC yet showed no nitrification capacity. This result indicates that, at least initially, organisms other than nitrifying bacteria were inhabiting the RBC discs.



LEGEND: RBC ——— 1-pH 7.5 – – – 2-pH 7.1
..... 3-pH 6.5 —·— 4-pH 6.3/6.7

Figure 4.5 RBC Disc Biofilm Development for the Low pH-Nitrification Study

Data on the development of ammonia-oxidizing and nitrite-oxidizing bacteria per unit disc area and per unit of dry volatile biofilm for each RBC are presented in Figures 4.6 and 4.7, respectively. The total number of viable nitrifying organisms reached an initial equilibrium in two to four weeks for all RBC systems. The biofilm populations for the pH 7.5 RBC and the pH 7.1 RBC reached an initial maximum in less than three weeks; however, the nitrification efficiency continued to improve throughout the first 30 days of operation. All the RBCs experienced population increases after the start of the PSU spring term. Figure 4.8 presents graphically the relative geometric mean data for the nitrifying bacterial populations per unit disc area and per unit volatile weight for each RBC system after the initial month of startup. These graphs demonstrate clearly that the total number of viable nitrifying bacteria on each RBC was related directly to overall RBC nitrification performance. The higher pH systems had larger populations of both ammonia-oxidizing and nitrite-oxidizing bacteria. The sustained depressed nitrification performance and the relatively low nitrifying bacteria populations of the 6.3/6.7 RBC indicated that a significant period of time was required for complete autotrophic adjustment in response to system changes under relatively low pH conditions. The ratios of ammonia-oxidizing bacteria to nitrite-oxidizing bacteria for the pH 7.5, 7.1, 6.5 and 6.3/6.7 RBC units were 16:1, 14:1, 9.4:1 and 3.3:1, respectively. This observation indicates that the lower pH systems favor nitrite-oxidizing bacteria relative to ammonia-oxidizing bacteria. This conclusion is shown graphically in Figure 4.8 which shows that the number of ammonia-oxidizing bacteria per dvg decreased with decreasing pH, while

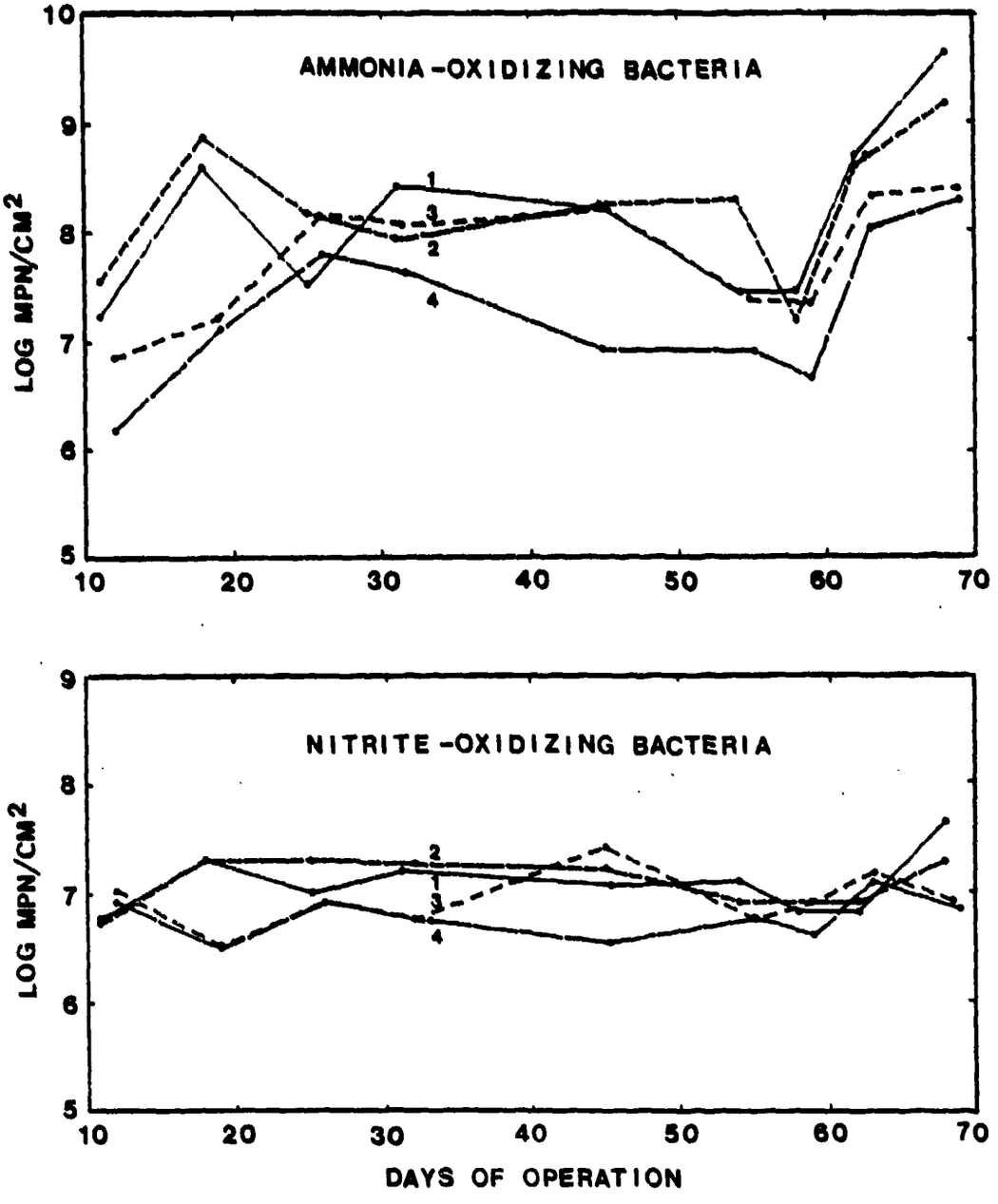


Figure 4.6 Development of Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria per Unit Disc Area for the Low pH-Nitrification Study

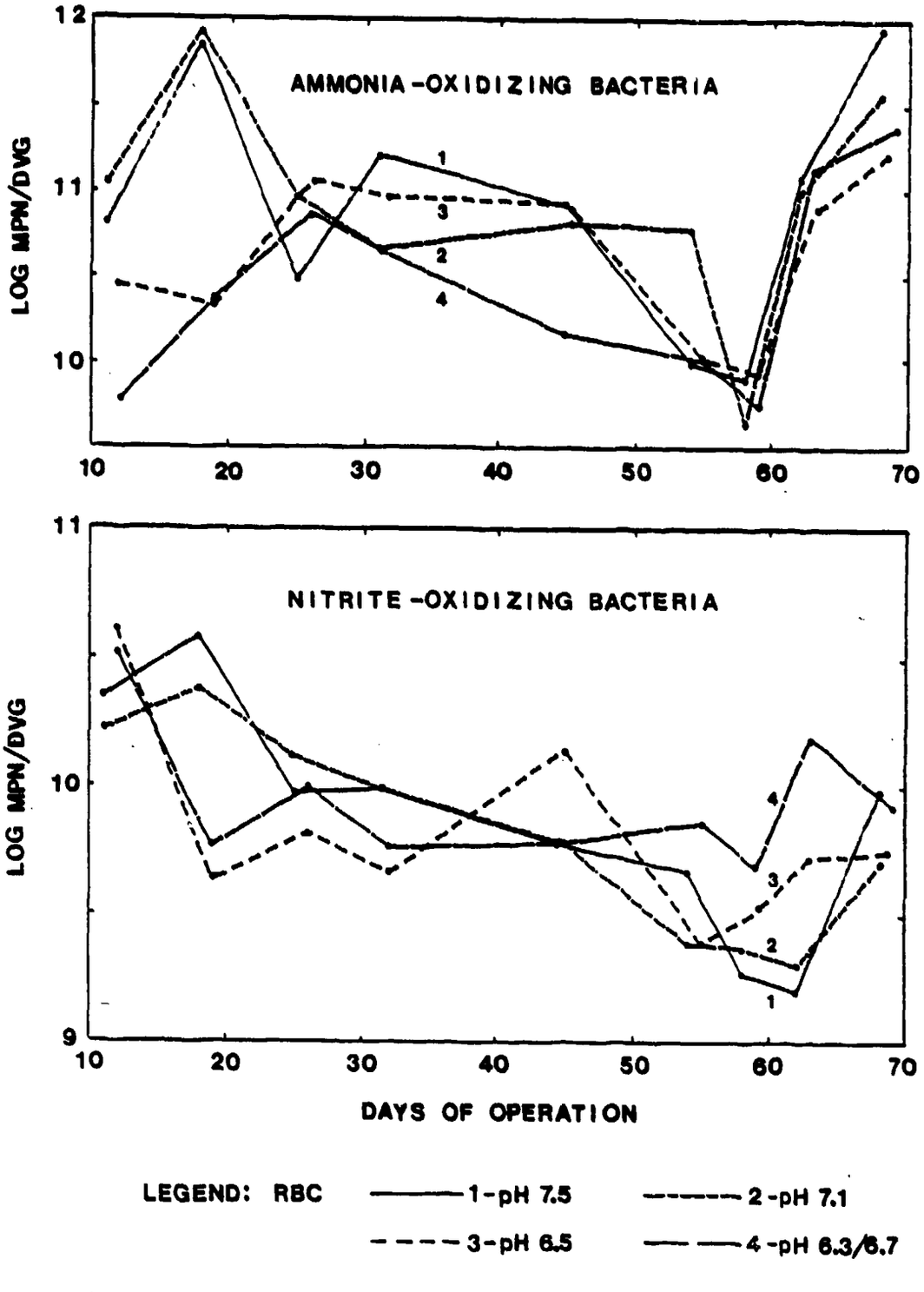
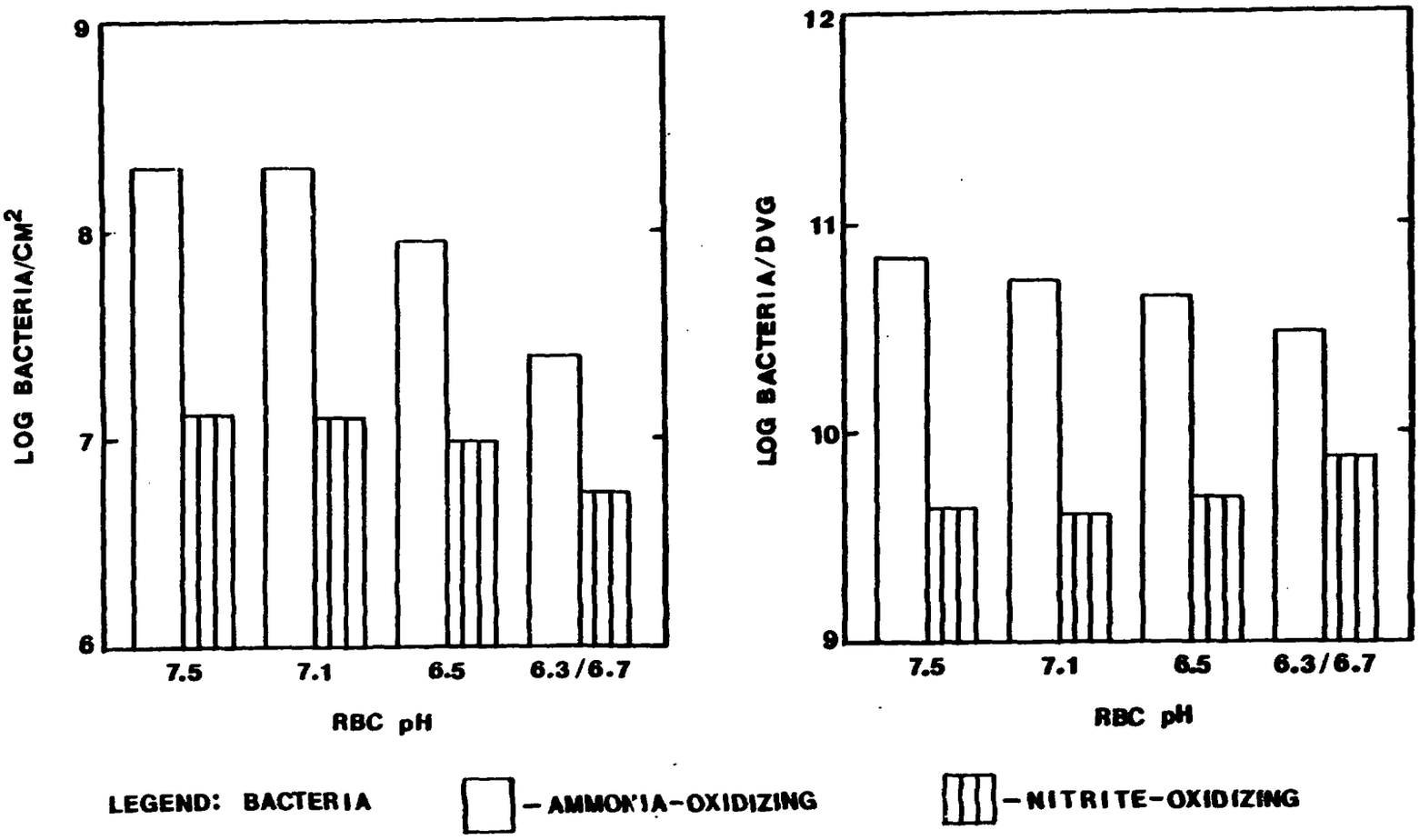


Figure 4.7 Development of Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria per Unit Dry Volatile Weight of Disc Biofilm for the Low pH-Nitrification Study



NOTE: The MPN data is taken from Figures 4.6 and 4.7. The initial month of MPN data is excluded from the geometric means.

Figure 4.8 Relative Geometric Mean Populations of Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria on a Unit Area and Unit Dry Volatile Weight Basis for the Low pH-Nitrification Study

the number of nitrite-oxidizing bacteria per dvg increased for the two lower pH RBC units. The volatile contents for these biofilms were 82, 83, 84, and 81 percent for the pH 7.5, 7.1, 6.5, and 6.3/6.7 RBCs, respectively.

Heterotrophic bacteria enumerations were performed between Day 58 and Day 68 of the study. The data were gathered over a relatively short period at the end of this study when the heterotrophic activity was enhanced by increased influent CBOD. The bacteria enumeration data are presented in Table 4.6.

Table 4.6 Heterotrophic Bacteria Enumerations for the Low pH-Nitrification Study[a]

RBC	pH	Cells $(10^{8})cm^{-2}$	Cells $(10^{11})dvg^{-1}$
1	7.5	4.2	1.3
2	7.1	3.6	1.1
3	6.5	4.0	2.0
4	6.3/6.7	1.9	2.4

[a]Enumerations presented are the geometric means from samples taken on Day 58, Day 62, and Day 68.

SECTION V

RBC NITRIFICATION OF HIGH RATE TRICKLING FILTER EFFLUENT

pH 7.6 - pH 8.8

5.1 Introduction

This research phase was devoted to the simultaneous evaluation of the relative rates of nitrification of domestic wastewater effluent from a high rate trickling filter within RBC systems maintained at normal and above normal wastewater pH levels. The four single stage nitrifying RBC systems used in the study treated the same influent wastewater and experienced the same cyclic variations in wastewater characteristics. The pH and alkalinity levels were adjusted upward artificially and maintained within three of the RBC systems. The fourth RBC system served as a control. The nominal hydraulic loading to each RBC system was 81 $l/m^2 \cdot d$ (2 $gal/d \cdot ft^2$). This research phase was 10 weeks in length. Operation of the RBC system then was extended 9 additional weeks for related substudies. The RBC systems were operated from 1 April, 1980 until 8 August 1980.

5.2 Experimental Apparatus and Procedures

The experimental equipment described in Section 4.2 was utilized in this high pH-nitrification study. This same configuration made it possible to observe simultaneously the relative rates of nitrification under varying pH and alkalinity levels while the other influent wastewater characteristics and operational conditions were common to all four RBC systems. Elevated pH and alkalinity environments were created by adding different concentrations of sodium hydroxide to three of the four

wastewater flow channels of the flow divider just prior to the flow entering into each of the completely mixed RBC systems. The sodium hydroxide addition points were changed to feed directly into the RBC units on Day 27 in order to provide better pH control and reduce solids generation. The sodium hydroxide concentrations were fed in this manner for the balance of the research. The fourth RBC treated the unaltered wastewater and served as the control. An ammonium chloride feed system was incorporated into the pilot facilities in order to prevent a severe ammonia depletion in the influent wastewater after the end of the PSU spring term. A schematic diagram of the pilot RBC systems is provided in Figure 5.1. The operational characteristics of the RBC systems are the same as provided previously in Table 4.1. Routine sampling of the four RBC influents and four RBC effluents was performed three days a week during startup and increased to five days a week during the nitrification equilibrium period. The sampling schedule was three and four days a week for the reversion and snail substudies, respectively. The sampling and analytical procedures described in Appendix A were utilized throughout this phase of the research.

5.3 Experimental Findings

5.3.1 Relative Rates of Nitrification

Data on the amounts of ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen entering and leaving each RBC throughout the first ten weeks of the study period are presented graphically in Figure 5.2. Data on the relative amount of ammonia removed by each RBC unit, as a function of time and pH, are presented in Figure 5.3. The initial rates of nitrification which developed during the first month, as shown in

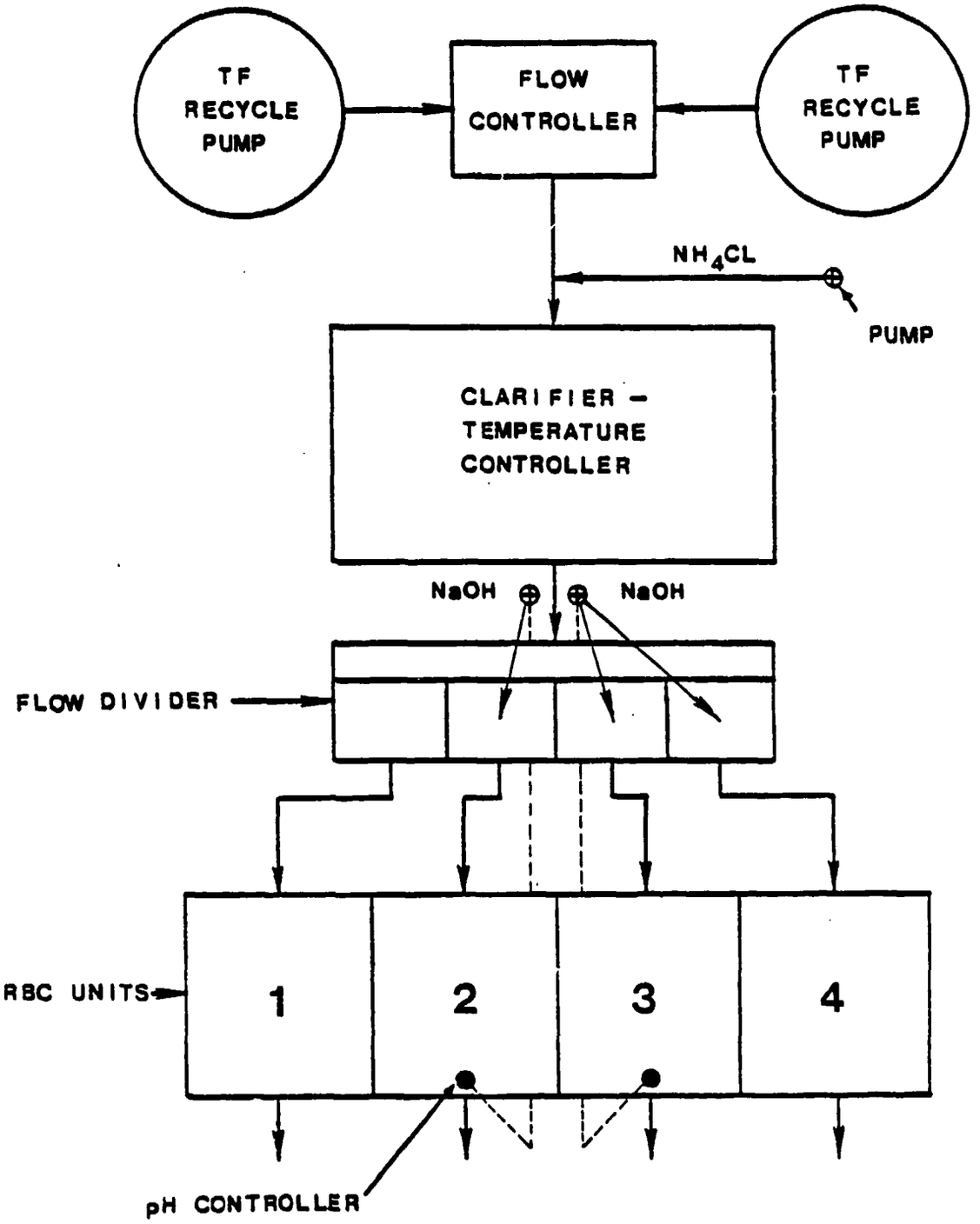


Figure 5.1 Schematic Diagram of the Pilot RBC Units for the High pH-Nitrification Study

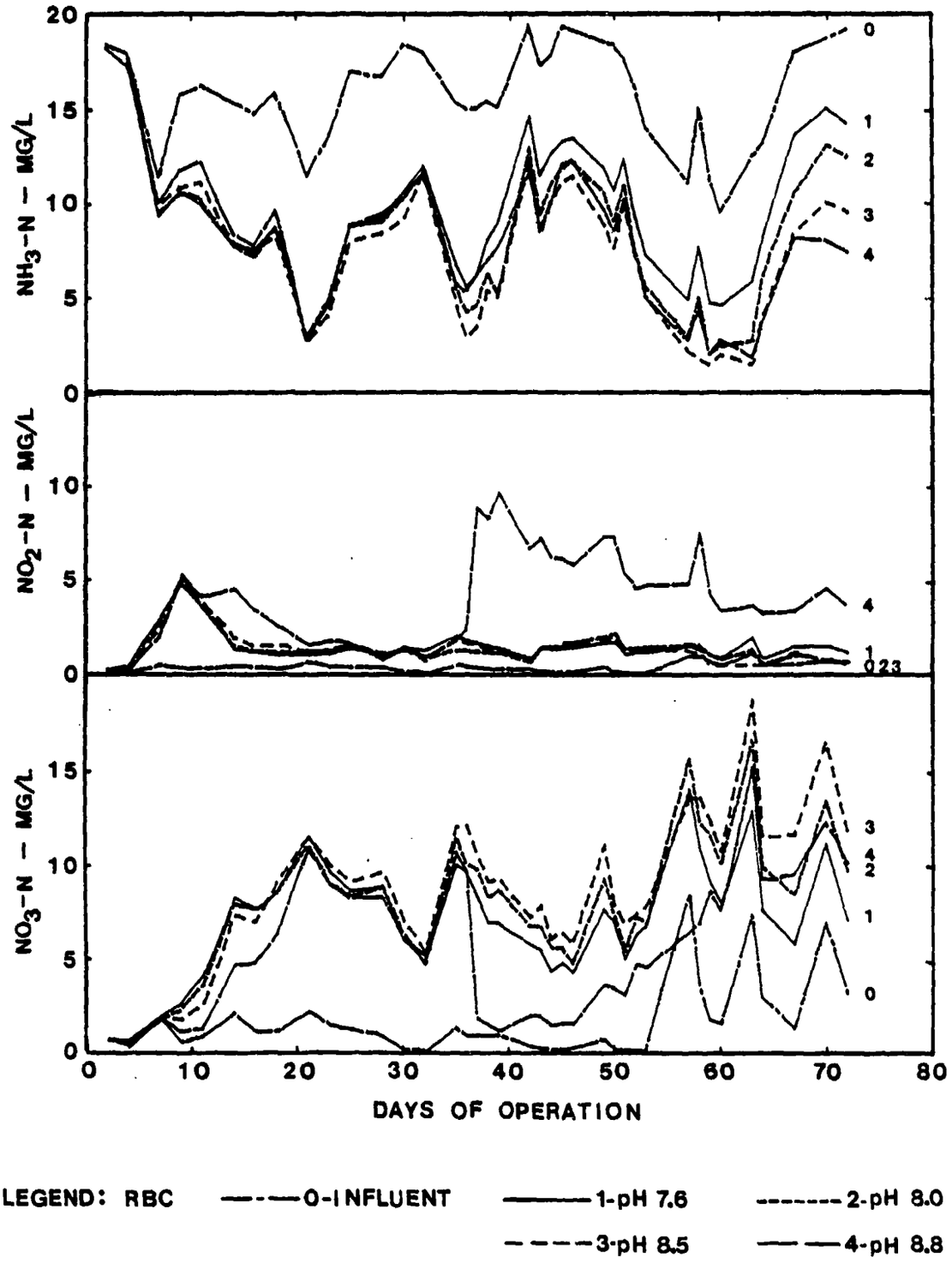


Figure 5.2 Influent and Effluent Nitrogen Forms for the RBC Units of the High pH-Nitrification Study

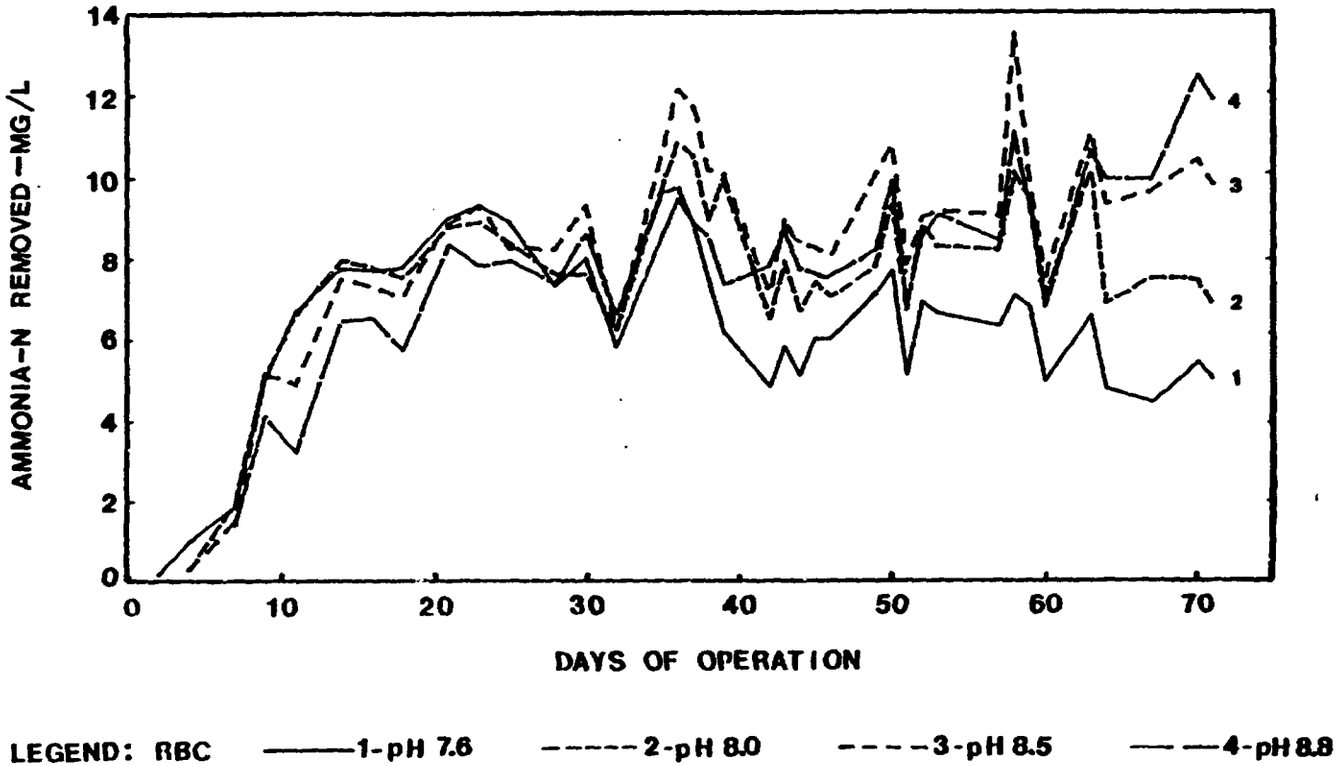


Figure 5.3 Relative Ammonia-Nitrogen Removal for the RBC Units of the High pH-Nitrification Study

Figure 5.3, were quite similar for the pH 7.6, 8.0, and 8.5 RBC systems. The relative rate for the pH 8.8 RBC developed more slowly. The control RBC operating at pH 7.6 developed a relatively high initial rate of nitrification, but its performance decreased with time. This control RBC followed the same trends as the pH 7.5 and pH 7.1 RBC units described previously in Section 4.3.1 for the low pH-nitrification study. However, the pH 8.5 RBC maintained the highest sustained level of nitrification performance throughout the entire ten-week period and did not decrease in performance as did the control RBC. After the initial month, the performance level of the pH 8.0 RBC was between that of the pH 7.6 RBC and the pH 8.5 RBC. The level of ammonia-oxidation for the pH 8.8 RBC fluctuated more widely than did the pH 8.0 RBC; however, its overall ammonia-oxidation performance was between that of the pH 8.0 and pH 8.5 RBC units. On day 36, a simultaneous flow stoppage and pH controller failure in the pH 8.8 RBC resulted in a pH excursion to approximately pH 11.0 for an estimated two hours within the RBC. The effect of this short-term transient condition produced dramatic and highly informative results. The data in Figure 5.2 demonstrate an immediate loss in nitrite-oxidation capability and a very slow nitrite-oxidation recovery. Interestingly, the data in Figure 5.2 and Figure 5.3 demonstrate that ammonia-oxidation for the pH 8.8 RBC hardly was affected. This indicates that the ammonia-oxidizing bacteria were much more resistant to elevated pH conditions than were the nitrite-oxidizing bacteria.

Based upon the ammonia removal, and not complete oxidation, a period of relative dynamic equilibrium was established after approximately five weeks of operation. A detailed comparison of the RBC units' influent and effluent wastewater characteristics, from Day 38 until Day

71 (the end of the initial 10 week period) is presented in Tables 5.1 and 5.2, respectively. Data on the relative amounts of ammonia-nitrogen removed by the four RBC systems during this period are presented in Table 5.3. The pH 8.8 RBC and the pH 8.0 RBC removed 94 and 84 percent as much ammonia as did the pH 8.5 system, respectively; whereas the control removed only 65 percent as much ammonia. Data on the nitrogen balances for the four RBC systems for this time period are presented in Table 5.4. The slightly lower nitrogen recoveries at the higher pH conditions may be due to small nitrogen losses resulting from ammonia stripping and denitrification within the heavier biofilms. This trend is similar to that trend noted previously in Section 4.3.1. These nitrogen recovery results indicate that ammonia stripping is not a major factor affecting the change in ammonia levels between pH 7.6 and pH 8.8.

The suspended solids level of the pH 8.8 RBC as noted in the data of Table 5.2 was higher than that of the other three units and showed a lower volatile content. This observation is attributed to low level precipitation of calcium carbonate. Figure 5.4 presents the mean CBOD removal data for the four RBC systems throughout the entire 19 weeks of the study. The influent CBOD level increased during the last weeks of the PSU spring term (Day 28 to Day 52) and then decreased sharply with the end of the term and remained low until the end of the study.

5.3.2 Biofilm Development and Microbial Enumerations

The RBC discs had no biofilm initially; however, the four RBC units developed biofilms which could be sensed by touch within 48 hours of startup. A noticeable reddish-brown biofilm was evident on all RBC discs on the third day of operation. By the eighth day, the four RBCs had developed thin and highly uniform textured biofilms which possessed

Table 5.1 RBC Influent Wastewater Characteristics for the High pH-Nitrification Study[a]

Parameter	RBC 1 (pH 7.6) Mean	Std. Dev.	RBC 2 (pH 8.0) Mean	Std. Dev.	RBC 3 (pH 8.5) Mean	Std. Dev.	RBC 4 (pH 8.8) Mean	Std. Dev.
Alk($CaCO_3$), mg/l	212(13)	42	219(11)	42	222(11)	42	222(12)	43
CBOD, mg/l	7.5(11)	3.9	10.2(8)	5.9	8.9(8)	3.2	9.1(8)	3.4
SS, mg/l	17(20)	4	18(19)	4	21(19)	7	20(19)	8
VSS, %	89(20)	4	88(21)	4	89(19)	4	89(19)	5
TKN-N, mg/l	19.4(21)	4.1	19.2(21)	3.7	19.3(21)	4.0	19.5(21)	3.7
NH_3-N, mg/l	16.1(21)	3.2	16.1(21)	3.2	16.2(21)	3.2	16.1(21)	3.2
Org-N, mg/l	3.3(21)	1.3	3.1(21)	1.0	3.1(21)	1.0	3.4(21)	1.3
(NO_2+NO_3)-N, mg/l	2.3(21)	2.9	2.3(21)	2.9	2.4(21)	2.9	2.3(21)	3.0
NO_2-N, mg/l	0.4(21)	0.3	0.4(21)	0.3	0.4(21)	0.3	0.4(21)	0.3
NO_3-N, mg/l	2.0(21)	2.6	2.0(21)	2.6	2.0(21)	2.6	1.9(21)	2.7
Flow, $l/m^2 \cdot d$	82(33)	5	84(33)	5	81(33)	5	82(33)	5

[a]Based upon data from Day 38 to Day 71.

[b]Number in parenthesis is the number of samples applied to statistical determinations.

Table 5.2 RBC Effluent Wastewater Characteristics for the High pH-Nitrification Study[a]

Parameter	RBC 1 (pH 7.6) Mean	Std. Dev.	RBC 2 (pH 8.0) Mean	Std. Dev.	RBC 3 (pH 8.5) Mean	Std. Dev.	RBC 4 (pH 8.8) Mean	Std. Dev.
pH	7.6(34)	-	8.0(34)	-	8.5(34)	-	8.8(34)	-
Alk($CaCO_3$), mg/l	169(15)	42	201(15)	49	231(15)	63	248(15)	67
CBOD, mg/l	5.7(11)	1.6	5.2(11)	2.3	4.9(12)	2.0	6.1(11)	1.9
SS, mg/l	18(21)	5	20(21)	6	23(21)	7	30(21)	14
VSS, %	89(21)	3	88(21)	4	85(21)	3	76(21)	7.4
TKN-N, mg/l	12.3(21)	3.9	9.9(20)	3.9	9.4(21)	4.1	9.7(21)	4.0
NH_3-N, mg/l	10.1(21)	3.5	8.0(21)	3.8	6.8(21)	3.6	7.2(21)	3.3
Org-N, mg/l	2.2(21)	1.3	2.1(21)	1.2	2.6(21)	0.8	2.6(21)	1.0
(NO_2+NO_3)-N, mg/l	8.8(21)	2.8	10.2(21)	3.3	11.2(21)	3.3	11.1(20)	3.1
NO_2-N, mg/l	1.3(21)	0.3	1.2(21)	0.4	1.1(21)	0.5	5.6(21)	1.8
NO_3-N, mg/l	7.5(21)	2.7	9.0(21)	3.3	10.1(21)	3.5	5.5(20)	4.2
DO, mg/l	5.2(30)	1.1	4.3(30)	0.9	4.1(30)	0.8	4.7(30)	0.6
Temp, ^oC	21.1(34)	1.1	21.1(34)	1.1	21.1(34)	1.1	21.1(34)	1.0

[a]Based upon data from Day 38 to Day 71.

[b]Number in parenthesis is the number of samples applied to statistical determinations.

Table 5.3 Relative Rates of Nitrification for RBC Systems Operating Under High pH Conditions[a]

RBC pH	Ammonia-N Removed ($g\ NH_3\text{-}N/m^2 \cdot d$)	Percent of Maximum
7.6	2.0	65
8.0	2.6	84
8.5	3.1	100
8.8	2.9	94

[a]Based upon data from Day 38 to Day 71.

Table 5.4 RBC Nitrogen Balances for the High pH-Nitrification Study[a]

RBC	pH	Total Nitrogen[b] - mg/l Influent	Effluent	Percent Recovery
1	7.6	21.7(21)[c]	21.1(21)	97
2	8.0	21.5(21)	20.2(21)	94
3	8.5	21.7(21)	20.6(21)	95
4	8.8	21.8(21)	20.8(21)	95

[a]Nitrogen balances are based upon data from Day 38 to Day 71.

[b]Total nitrogen is total oxidized nitrogen plus total Kjeldahl nitrogen (TKN).

[c]Number in parenthesis is the number of samples utilized in the total nitrogen evaluations.

a visually apparent gradation. The pH 8.5 and pH 8.8 RBC biofilms initially developed more rapidly than the pH 7.6 and pH 8.0 RBC biofilms. This initial biofilm gradation was not related to ammonia removal efficiency. The biofilm color had changed from reddish-brown to tan or bronze on all discs by the 10th day. By the 13th day of operation, the

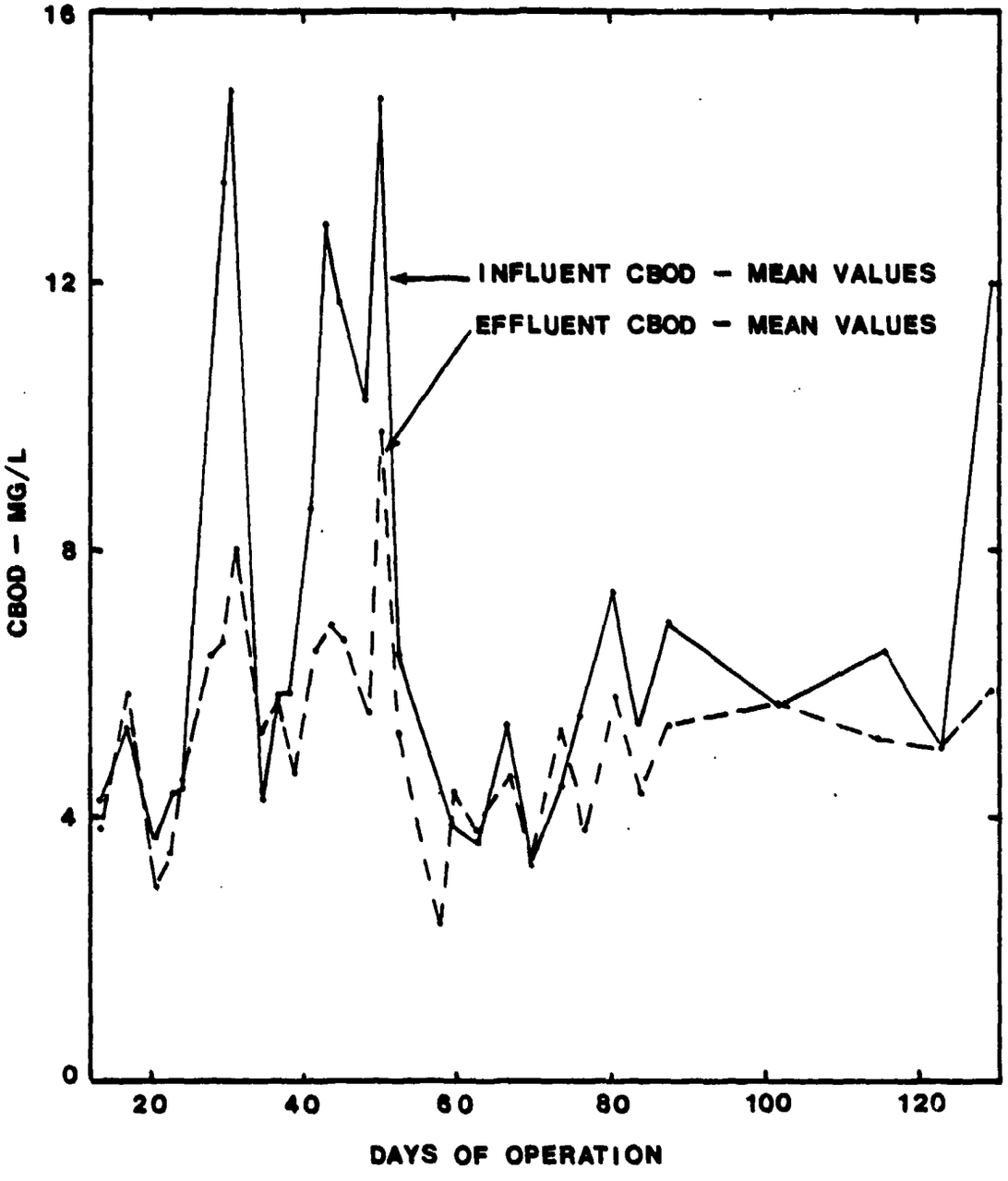


Figure 5.4 Influent and Effluent CBOD for the RBC Units of the High pH-Nitrification Study

two lower pH RBCs had the most uniformly textured biofilms while the pH 8.5 and pH 8.8 RBCs were developing a "dimpled" appearance associated with the heavier biofilms. The pH 8.8 RBC developed a patchy appearance and also had started to slough significantly after only two weeks. As time progressed, the RBC systems added biofilm, but their texture became less uniform. The heavier biofilms appeared to be associated with the pH 8.0 and pH 8.5 systems. The pH 8.8 RBC experienced the greatest biofilm sloughing even before the upset condition described in Section 5.3.1 above. After the end of the spring term, a marked reduction in biofilm in all four systems was noted. The photograph in Figure B.2, Appendix B, qualitatively describes the varying biofilm characteristics for the four RBC systems at the end of the ten-week study period. In general, during the later half of this period, the size of the suspended particulates within the pH 8.8 RBC trough was smaller than that of the suspended solids in the other three RBC units which had more biofilm attached to the discs. Snails first were noted in the troughs of the pH 7.6 and pH 8.0 RBC systems on Day 22. They increased in number more rapidly in the pH 7.6 RBC than in the pH 8.0 RBC; however, they never inhabited any of the rotating discs. Snails did not take up residence in either the pH 8.5 or pH 8.8 RBC systems.

Figure 5.5 presents the RBC disc biofilm development data for all four RBC systems during the high pH-nitrification study. After the initial month of operation, the levels of disc biofilm for the pH 7.6, 8.0, and 8.5 RBCs were related directly to their relative level of performance. The pH 8.8 RBC experienced an initially high rate of biofilm development; however, it reached a peak mass per unit area concentration on Day 31 and then experienced a continuous biofilm loss. All four RBC

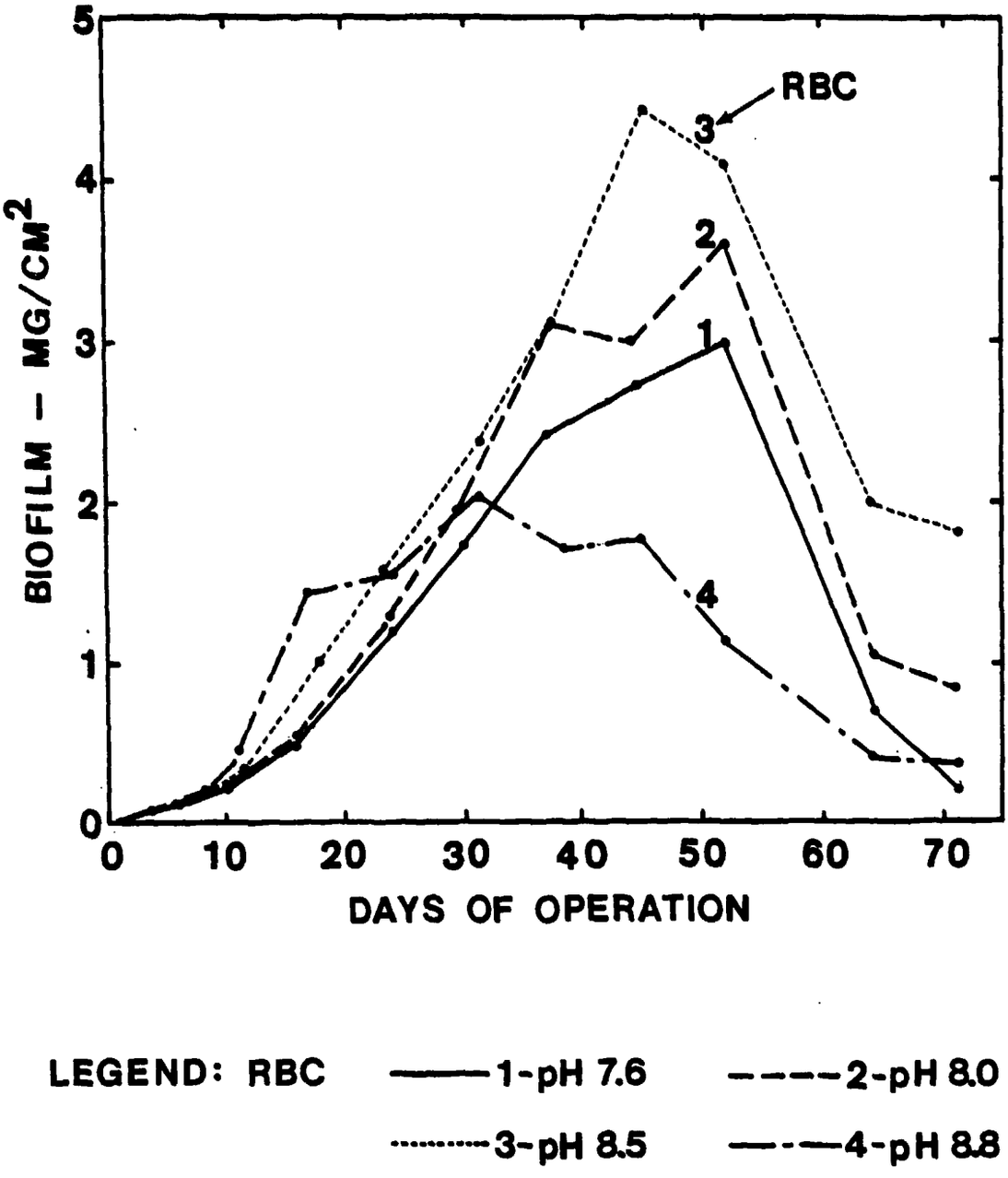


Figure 5.5 RBC Disc Biofilm Development for the High pH-Nitrification Study

systems experienced a marked decline in disc biofilm after the end of the PSU spring term on Day 52 when the influent CBOD concentration decreased. The four RBC systems appeared to achieve an initial maximum level of performance in about three weeks. These performance levels corresponded to disc biofilm concentrations of approximately 0.8, 1.0, 1.2, and 1.4 mg/cm^2 for the pH 7.6, 8.0, 8.5, and 8.8 RBCs, respectively. As demonstrated previously in Sections 3.3.2 and 4.3.2, the increase in disc biomass did not improve the rate of nitrification for any of the systems.

Data on the development of ammonia-oxidizing, nitrite-oxidizing, and heterotrophic bacteria per unit of disc area and per unit weight of dry volatile biofilm for each RBC are shown in Figures 5.6 and 5.7, respectively. In general, the populations of all three groups of organisms continued to increase for the initial 30 days of biofilm development. The heterotrophic population developed most rapidly in the pH 8.5 and pH 8.8 RBC systems.

A general downward trend for all three groups of organisms started with the end of the PSU spring term on Day 52. The pH 8.8 RBC experienced a pronounced decline in the population of nitrite-oxidizing bacteria shortly after the occurrence of the pH upset cited in Section 5.3.1 above. Simultaneous decreases in the numbers of ammonia-oxidizing and heterotrophic bacteria for the pH 8.8 RBC were not observed. This observation correlates well with the RBC's loss of nitrite-oxidization capacity while retaining the ability to oxidize ammonia. Figure 5.8 presents graphically data on the relative geometric mean bacteria populations per unit of disc area and per unit weight of dry volatile biofilm for each RBC system after the initial 30-day period. The total

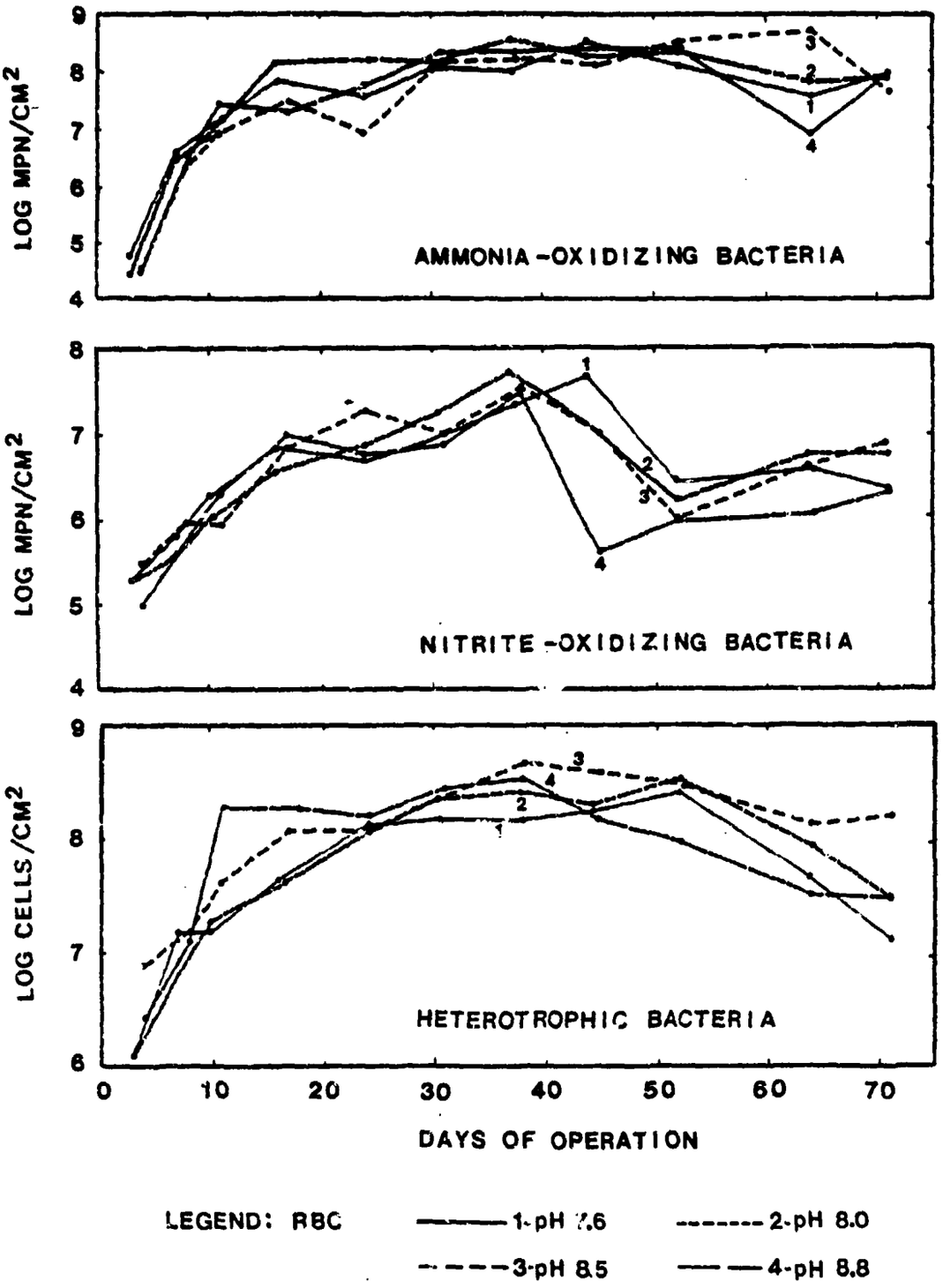


Figure 5.6 Development of Ammonia-Oxidizing, Nitrite-Oxidizing, and Heterotrophic Bacteria Per Unit of Disc Area for the High pH-Nitrification Study

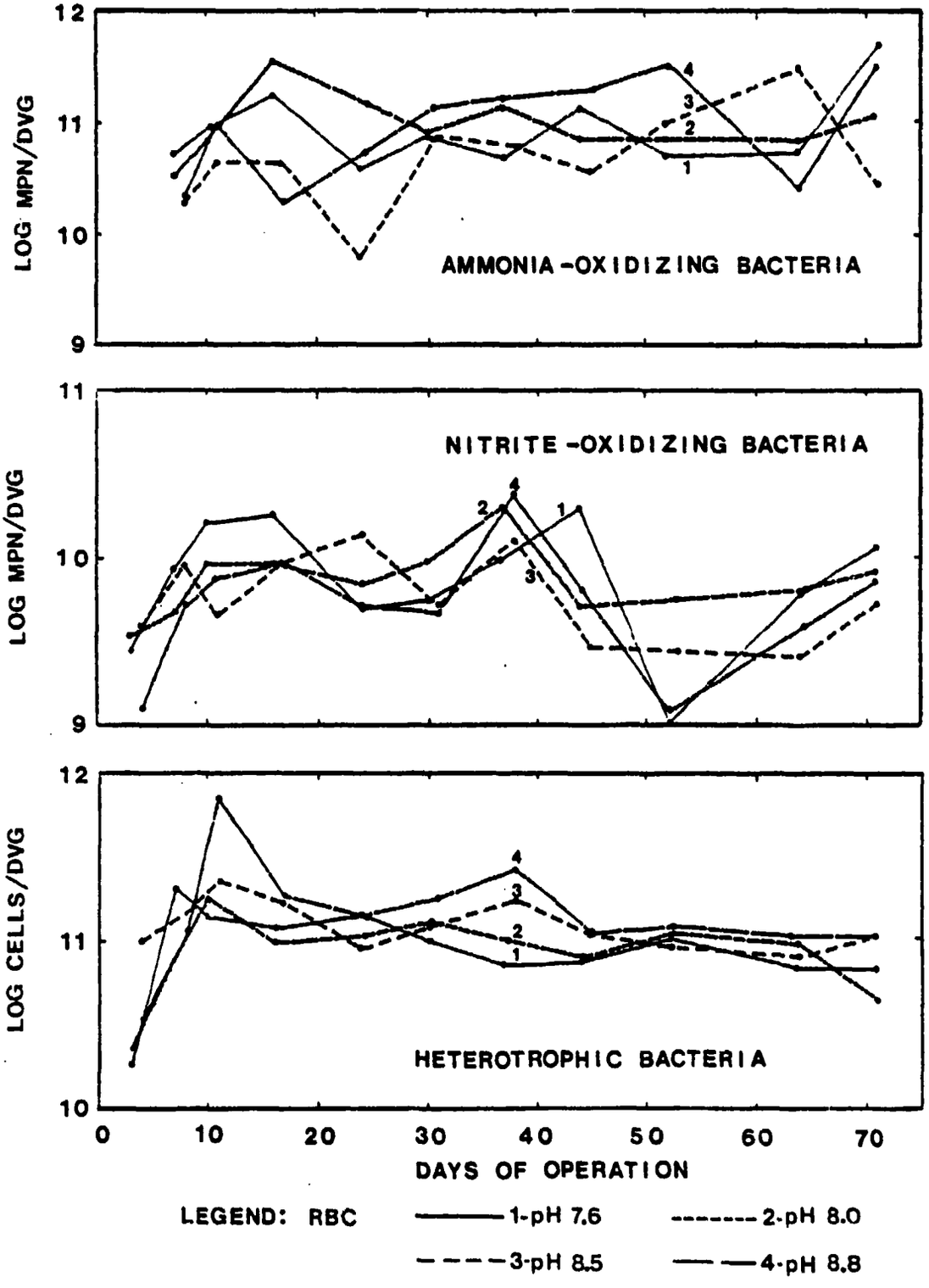
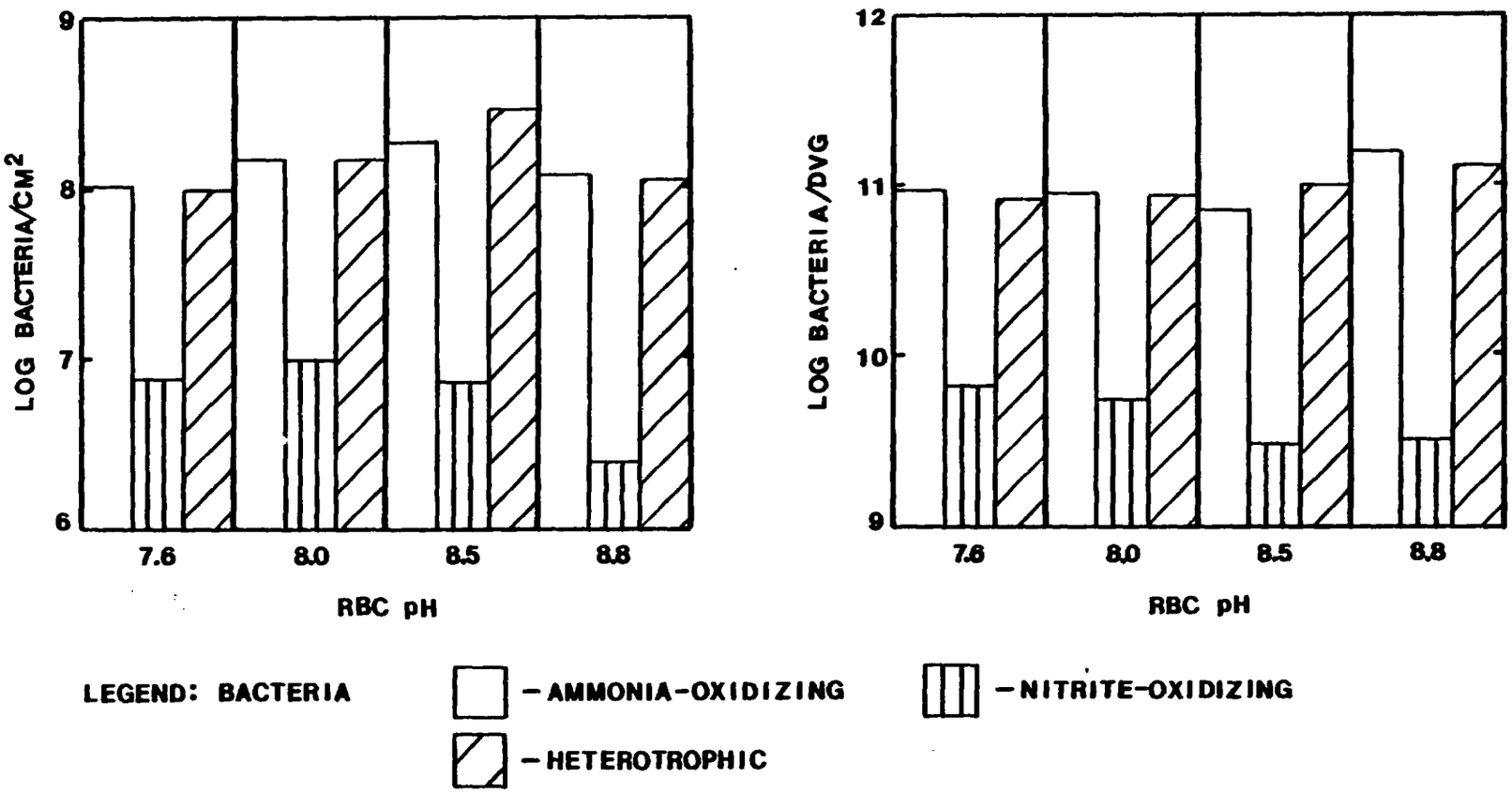


Figure 5.7 Development of Ammonia-Oxidizing, Nitrite-Oxidizing, and Heterotrophic Bacteria Per Unit Weight of Dry Volatile Disc Biofilm for the High pH-Nitrification Study



NOTE: Geometric means are based upon six sets of enumerations for each bacteria classifications from Day 30 to Day 71. Nitrifying enumerations are MPN values and heterotrophic enumerations are plate counts.

Figure 5.8 Relative Geometric Mean Populations of Ammonia-Oxidizing, Nitrite-Oxidizing, and Heterotrophic Bacteria on a Unit Area and Unit Weight of Dry Volatile Disc Biofilm Basis for the RBCs of the High pH-Nitrification Study

numbers of ammonia-oxidizing and heterotrophic bacteria increased with increasing pH up to pH 8.5 and then experienced a drop at pH 8.8. The total number of nitrite-oxidizing bacteria was greatest at pH 8.0 and decreased at pH 8.5 and pH 8.8. The number of ammonia-oxidizing bacteria relative to the total biofilm population was similar for the pH 7.6, 8.0, and 8.5 systems but greatest at pH 8.8. Similarly, the heterotrophic population increased with pH. However, the nitrite-oxidizing bacteria populations were nearly identical at pH 7.6 and pH 8.0 but lower at pH 8.5 and pH 8.8. The ratios of heterotrophic to ammonia-oxidizing to nitrite-oxidizing bacteria in Figure 5.8 for the pH 7.6, 8.0, 8.5, and 8.8 RBC units were 12:15:1, 16:16:1, 37:24:1, and 43:48:1, respectively. In general, these population figures reveal that the heterotrophic bacteria and the ammonia-oxidizing bacteria are favored over the nitrite-oxidizing bacteria with respect to increasing pH. During this period, the mean biofilm concentrations were 1.80, 2.26, 2.99, and 1.23 mg/cm^2 for the pH 7.6, 8.0, 8.5, and 8.8 RBC units, respectively. The volatile content was 86, 86, 83, and 75 percent for the pH 7.6, 8.0, 8.5, and 8.8 RBC units, respectively. The increase in inert material within the pH 8.8 RBC biofilm was similar to the increase in inert suspended solids within the pH 8.8 RBC noted previously in Section 5.3.1. This lower volatile content was attributed to low level precipitation of calcium carbonate and entrainment within the disc biofilm.

5.3.3 Short Term pH Effect on Nitrification

At the conclusion of the 10-week high pH-nitrification study, the two RBC systems which had been operating at pH 7.6 and pH 8.5 were utilized in a short term pH-nitrification study wherein the two RBC

systems experienced simultaneous short term changes in pH, i.e. 2 hours (four trough volume turnovers) of operation at each pH level. The pH level started at pH 9.0 and was decreased progressively downward to pH 6.0 without interruption. Alkalinity and pH levels were maintained in each RBC by direct feed of sodium hydroxide and sulfuric acid solutions. This test was run twice (Day 73 and Day 79) with similar results. The average values from these two runs are shown in Figure 5.9.

The shapes of the performance curves for the two RBCs are similar yet the level of nitrification for the two RBCs are markedly different. Clearly, the RBC which had acclimated at pH 8.5, and had a history of elevated performance, retained its higher performance level in the short term and continued to perform significantly better than the biofilm acclimated at pH 7.6. The RBC response to short term changes in pH is relatively constant between pH 7.0 and pH 9.0 but highly dependent upon the previous acclimated level of nitrification for the given RBC biofilm. Data on the alkalinity levels have been included in Figure 5.9. The amount of alkalinity present at the low pH levels demonstrates that even at 20 mg/1 of $CaCO_3$, significant amounts of nitrification were achieved. The response of the lower pH system closely resembles the data of Borchardt (16) and similarly reveals good nitrification at very low alkalinity levels. This result tends to reinforce the observation that the pH level is much more important than the alkalinity level per se in terms of effect on nitrification.

5.3.4 Nitrification Reversion Study

The long term pH-nitrification studies had been successful in demonstrating differences in biofilm and RBC performance as a function

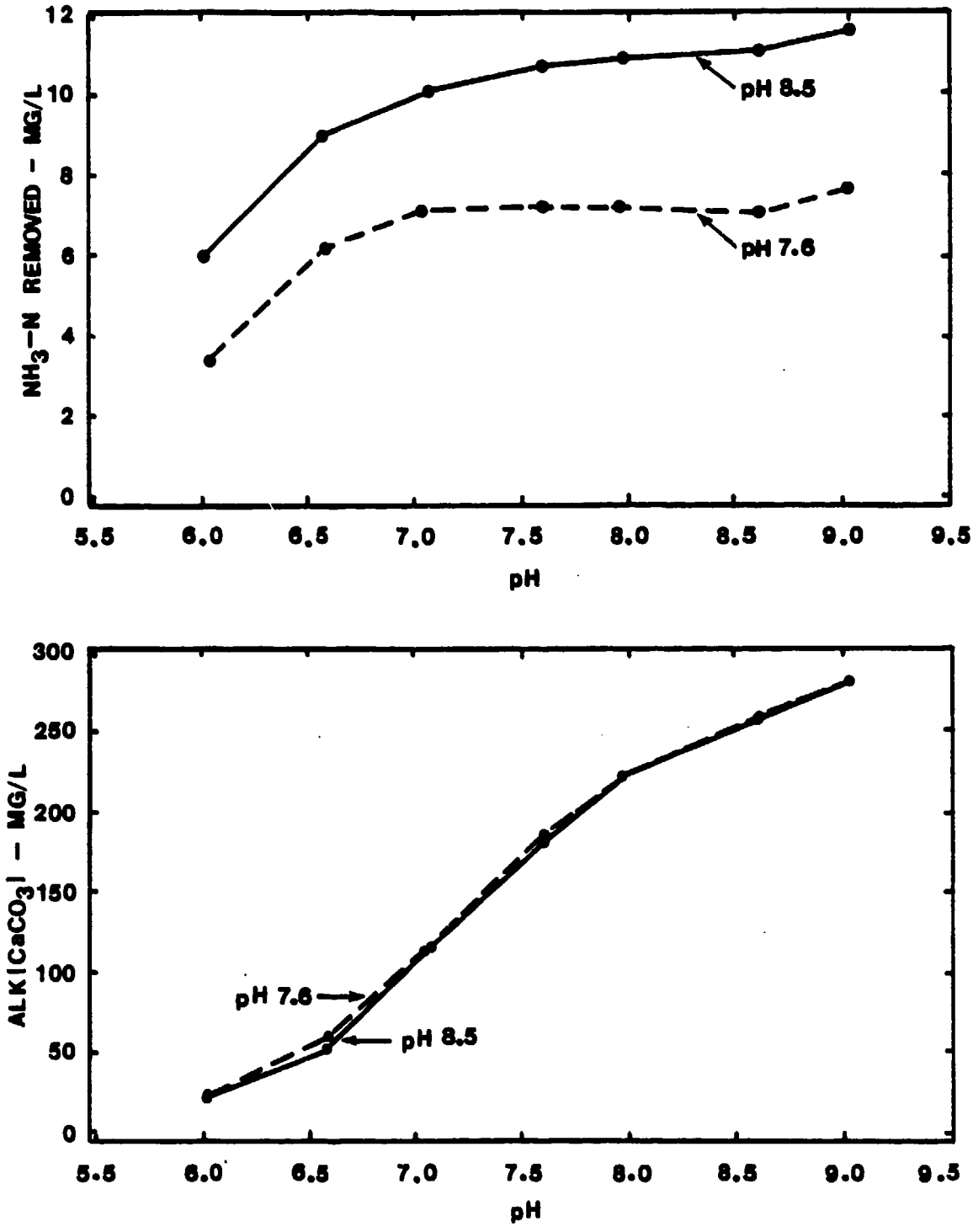


Figure 5.9 The Relative Rates of Nitrification of RBC Systems Acclimated at pH 8.5 and pH 7.6 and Subjected to Short Term pH Changes and Related Alkalinity Levels

of pH. This substudy was performed to evaluate the length of time it would take for the RBC nitrification performance to readjust, or revert, to that performance level developed at a lower pH condition. In order to evaluate the length of time for this reversion to take place, the pH control for the pH 8.0 and pH 8.8 RBC units was terminated on Day 71. The control and pH 8.5 RBC units continued operating as before. The resulting data are presented in Figure 5.10. The nitrifying performance of RBC 2, which had been operating at pH 8.0, remained high for approximately one week at which time major sloughing was noted, and the performance deteriorated to a level below that of the pH 7.6 unit. This experience is very similar to that reported by Miller (71). The performance level of RBC 2 then dropped significantly below that of the control for approximately five weeks. The performance of the RBC unit which had operated at pH 8.8, took longer to revert to the level of the control RBC. Reversion for this system took 19 days. During the 39 days of the reversion study the pH 8.5 RBC and the control RBC removed 9.9 mg/l and 8.0 mg/l of ammonia-nitrogen, respectively. The control RBC removed 81 percent as much ammonia as the pH 8.5 RBC.

5.3.5 Effect of Snail Populations on RBC Nitrification

Snails which infested the PSU high-rate trickling filters were transported in the filter effluent wastewater and eventually inhabited those RBC units operated at or below pH 8.0. The appearance of snails was due initially to physical migration of snails into the RBC units. However, once in the RBC, the snails actually proliferated within the RBC trough by depositing gelatinous egg sacks on the trough walls. The snails harvested RBC trough wall biofilm but did not occupy the rotating

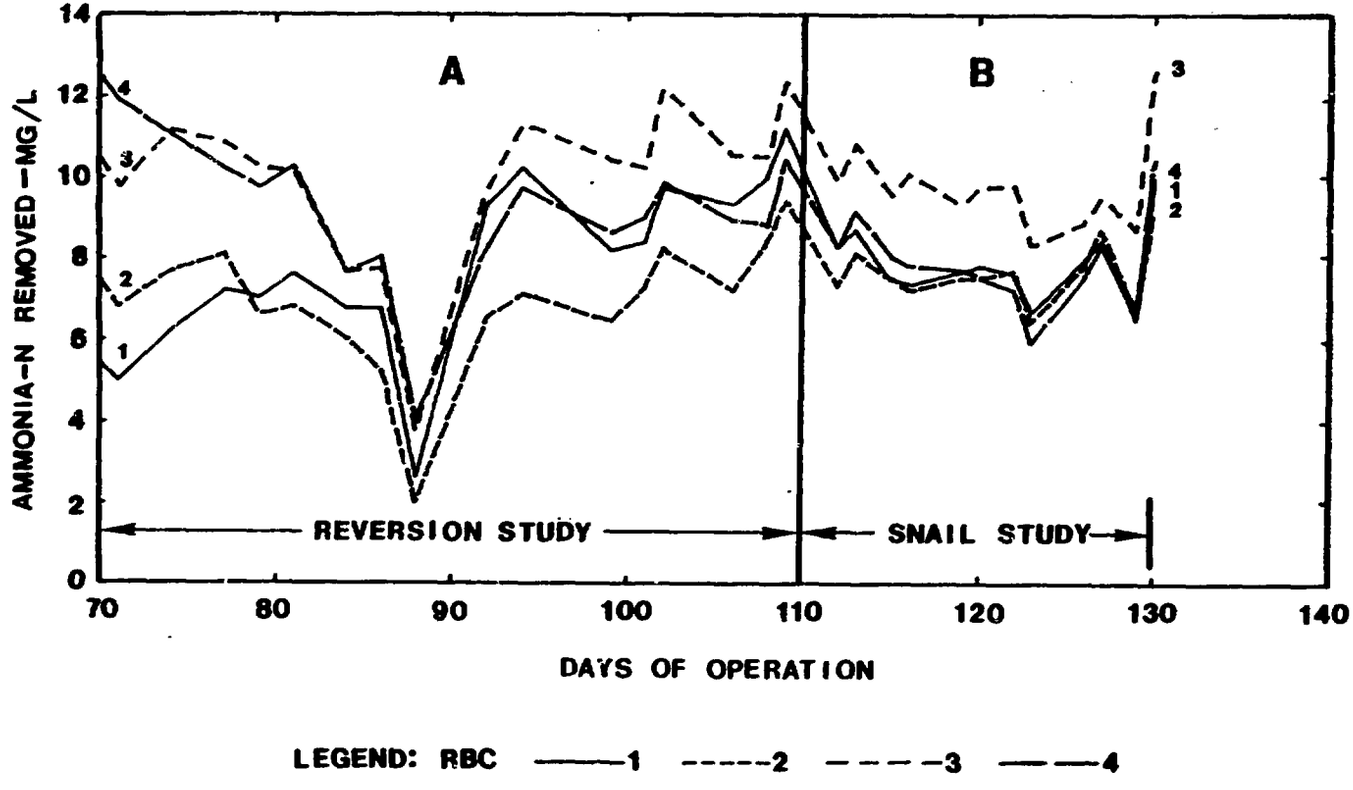


Figure 5.10 (a) Nitrification Reversion of RBC Systems Acclimated at pH 8.0 (RBC 2) and pH 8.8 (RBC 4)
(b) Comparison of the Nitrification Performance of an RBC System Operating Without Snails (RBC 4) Against Two Controls Operating with Snails (RBC 1 and 2) and the pH 8.5 (RBC 3)

discs. The existence of the snails in the RBC troughs raised the question as to their impact, if any, on the nitrification process. The effect of harvesting wall growth was unknown, but the impact was considered minimal because the wall biofilm only constitutes approximately nine percent of the total RBC surface area. In addition, the rounded shells of the snails provided additional surface area for biofilm attachment.

On Day 110, RBC 4, which had been operating at pH 7.6 and had been undergoing reversion since Day 71, was purged of snails by elevating the pH to 8.5 for one hour which caused the snails to migrate out of the bulk solution of the RBC trough. The snails then were harvested along the waterline. Each day thereafter, the remaining snails and new snails arriving in the influent were scraped from slightly below the trough waterline where they would reside normally when inhabiting the trough in small numbers. The nitrification performance of the snail free RBC was related to the performance of the pH 8.5 RBC and the original control, as well as to the RBC unit which formerly had been operated at pH 8.0, and also had been undergoing reversion at pH 7.6 since Day 71. The ammonia removal performance data of the four systems are shown in Figure 5.10. Any improvement in nitrification resulting from the loss of snails and increased wall biofilm should have been observable in two to three weeks. On Day 130, the experiment was terminated when it became evident that the snail free RBC was nitrifying at essentially the same rate as the two control RBC units. During this 20 day period the pH 8.5 RBC and the control RBC removed 9.8 mg/l and 7.9 mg/l of ammonia-nitrogen, respectively. The control RBC removed 80 percent as much ammonia as the pH 8.5 RBC.

5.3.6 RBC Hydraulic Tracer Study

The hydraulic characteristics of the four RBC units were examined by conducting a hydraulic tracer study on each RBC with biofilm on the discs. The four tests were conducted simultaneously on Day 116. Sodium chloride, which was used as the tracer was added to the flow divider preceding the RBCs. The results of the tracer study are presented in Figure 5.11 and Table 5.5. The data reveal that none of the four RBC systems was subjected to severe short circuiting and that performance variations between RBCs could not be explained by differing hydraulic characteristics.

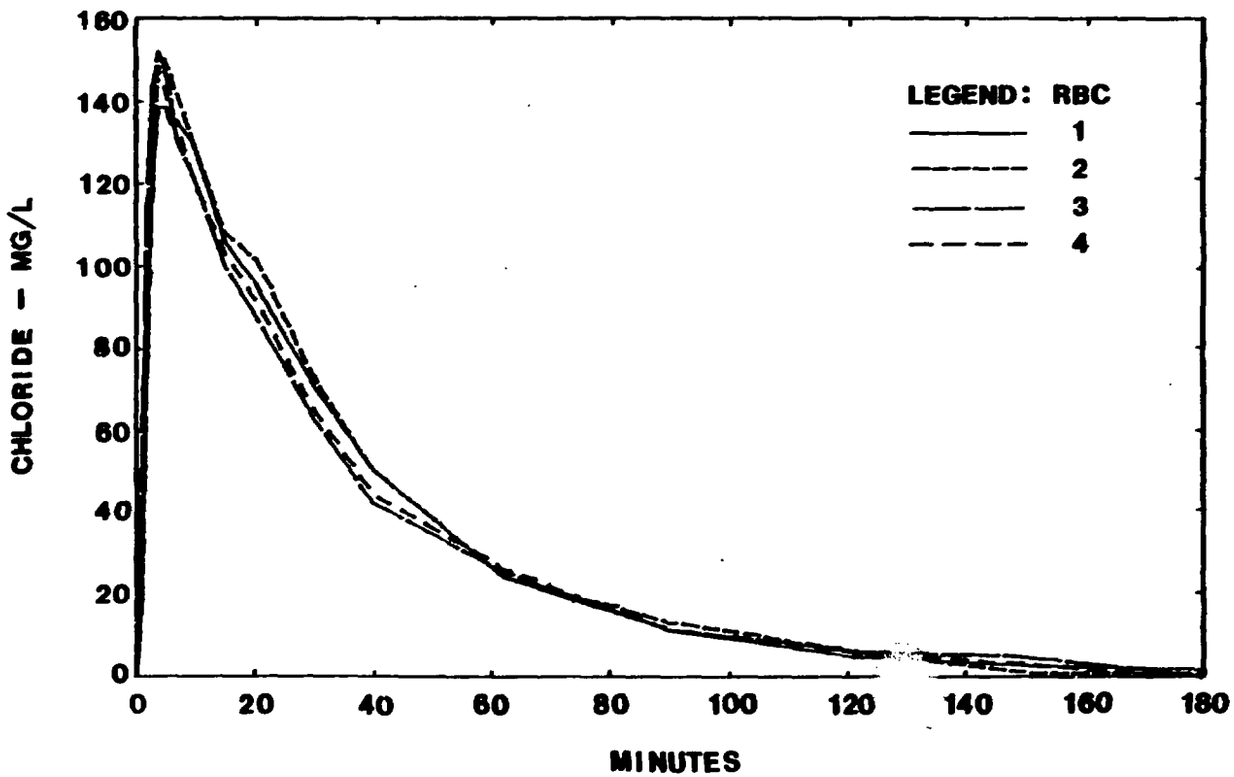


Figure 5.11 Simultaneous Hydraulic Tracer Studies of the Four RBC Units With Biofilm

Table 5.5 Hydraulic Characteristics of the Four RBC Systems

Parameter	RBC 1	RBC 2	RBC 3	RBC 4
Flow Rate, lpm	1.21	1.18	1.25	1.20
RBC Vol., l	35	35	35	35
T = V/Q, min.	31	31	30	31
T_p[a], min.	4.5	5.0	4.0	4.0
T_{10}[b], min.	6	6	6	6
T_{50}[c], min.	26	25	26	26
T_{90}[d], min.	85	88	95	90
Disc Biofilm, mg/cm^2	0.83	2.51	3.75	1.36
Tracer Recovery[e], %	102	102	102	99

a. T_p = time for tracer peak.
b. T_{10} = time for 10 percent of total tracer.
c. T_{50} = time for 50 percent of total tracer.
d. T_{90} = time for 90 percent of total tracer.
e. Tracer was sodium chloride.

SECTION VI

RBC NITRIFICATION ENHANCEMENT THROUGH ALKALINE CHEMICAL ADDITION

6.1 Introduction

This research phase was devoted to the simultaneous evaluation of the nitrification of high rate trickling filter domestic wastewater effluent within RBC systems maintained at elevated pH levels through alkaline chemical addition. Five 2-stage nitrifying RBC systems operating in parallel simultaneously treated the same wastewater thereby experiencing the same cyclic variations in influent wastewater characteristics. The pH and alkalinity levels within four separate RBC systems were adjusted upward and maintained artificially with four different alkaline chemicals. The fifth RBC system served as a control. The nominal hydraulic loading to each RBC system was 81 $l/m^2 \cdot d$, (2 $gal/d \cdot ft^2$). This research phase was approximately 11 weeks in length. The study was extended 4 additional weeks to compare the nitrification performance of 1-stage against 2-stage alkaline chemical addition. The RBC systems were operated from 8 September until 19 December, 1980.

6.2 Experimental Apparatus and Procedures

The effluent from the PSU WWTP high rate trickling filters, Figure 2.1, passed through a flow controller, a plexiglass secondary clarifier, and a flow divider which split the wastewater flow equally into five separate channels. The five wastewater channels passed into the five 2-stage RBC systems. The first stages of four of the RBC systems received an alkaline chemical for pH control. For the purpose of this

study, each individual stage of a common 4-stage 0.5 meter diameter pilot RBC was used as the first stage of a 2-stage system. Similarly, the second stages were the individual stages of a second 4-stage 0.5 meter diameter pilot RBC which operated in series with the first RBC unit. The control RBC system was a separate 2-stage 0.5 meter pilot RBC with identical geometry and the same serpentine hydraulic flow pattern as the other four 2-stage RBC systems. The discs of all five RBC systems were assembled from random selections of discs taken from three 0.5 meter pilot scale RBC systems. This procedure was followed to ensure that disc surface characteristics associated with age and history of use would not be a factor. Disc extenders were added to all stages to enhance trough turbulence and minimize potential solids accumulation in the troughs.

In order to simulate a low pH-low alkalinity wastewater, sulfuric acid was added between the flow controller and the clarifier. The wastewater entering each RBC system was at pH 6.5 and contained approximately 150 mg/l of alkalinity. The pH and alkalinity of four of the RBC systems then were adjusted upward through the addition of sodium hydroxide (caustic), sodium carbonate (soda ash), calcium hydroxide (lime), and sodium bicarbonate. The alkaline chemicals were fed directly into the first stage of the respective RBC systems. The pH levels in the first stage of the sodium hydroxide, sodium carbonate, and calcium hydroxide RBC systems were maintained at approximately pH 8.5. This pH had been found to be the optimum pH for RBC nitrification as reported previously in Sections IV and V. Sodium bicarbonate was incapable of raising the pH above 8.3 and, therefore, the first stage of the sodium bicarbonate system arbitrarily was maintained at an intermediate pH level of pH 7.5.

The control RBC system treated the low pH-low alkalinity wastewater. An ammonium chloride feed system was incorporated into the pilot plant facilities in order to prevent a severe ammonia depletion of the influent wastewater during the PSU Thanksgiving holiday break. A schematic diagram of the pilot RBC systems is shown in Figure 6.1. The operational characteristics of the RBC systems are presented in Table 6.1. Routine sampling of the secondary clarifier effluent and the effluent from each RBC stage was performed three days a week during startup and five days a week once nitrification equilibrium was achieved. All sampling and analytical procedures used are described in Appendix A.

6.3 Experimental Findings

6.3.1 Relative Rates of Nitrification

The data on the amounts of ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen entering each of the five RBC systems from Day 1 to 75 are presented in Figure 6.2. This time period coincides with the entire 1980 PSU spring term. Figure 6.3 presents the data describing the relative amounts of ammonia-nitrogen removed by each of the five RBC systems for this same period. The data on the ammonia removed for each RBC stage are shown in Figures 6.4 and 6.5. The overall nitrification capacity of the 2-stage RBC control system treating the low pH-low alkalinity wastewater developed more slowly than did that of the higher pH systems. This difference was due to the fact that the first stage of the control RBC developed its nitrifying capacity more slowly than did the second stage of the control RBC. The more rapid development of the second stage nitrification capacity may have been due to the removal of much of the influent CBOD within the first stage and lessening of the heterotrophic

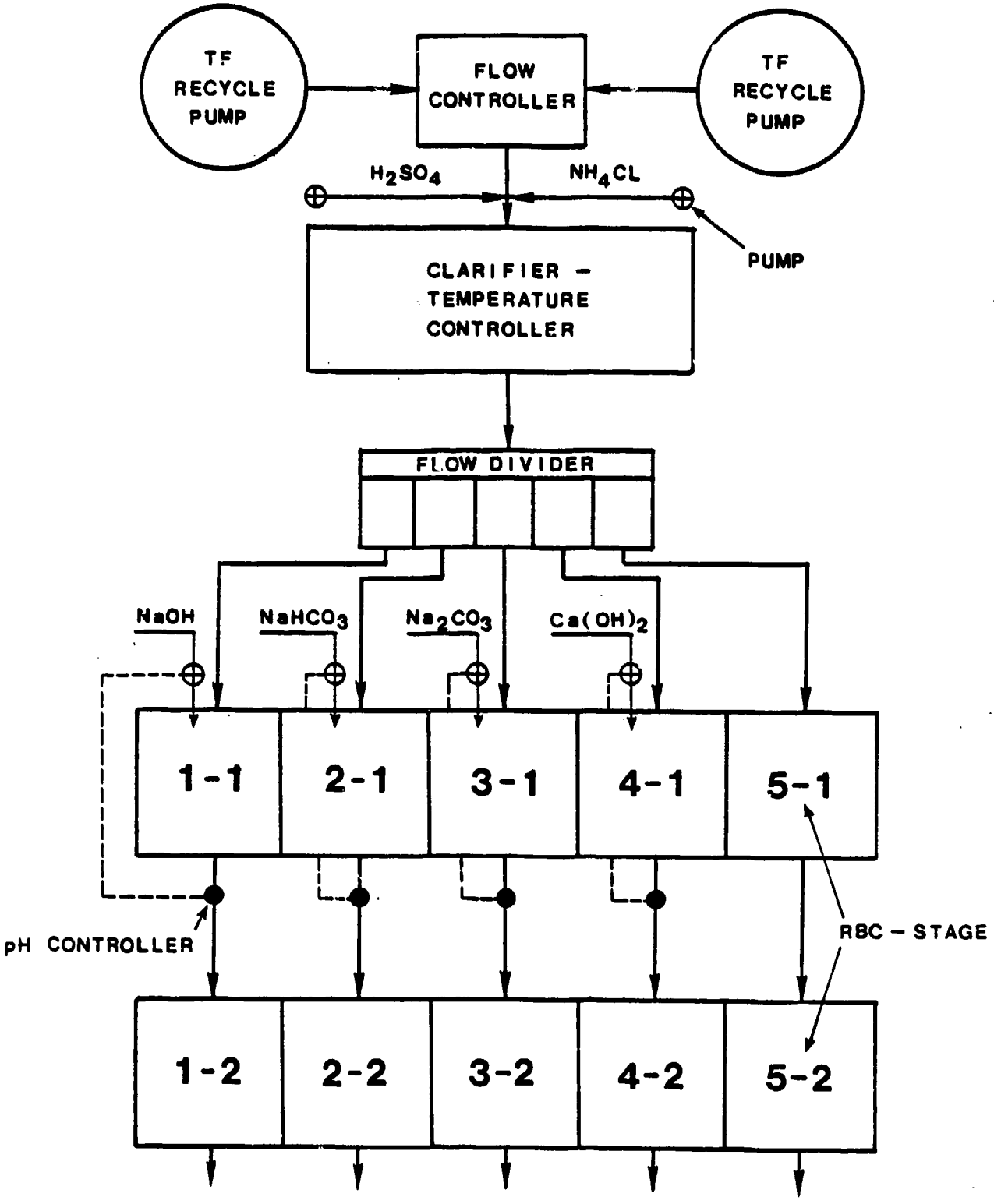


Figure 6.1 Schematic Diagram of the 2-Stage RBC Systems of the Alkaline Chemical Addition Study

Table 6.1 Pilot 2-Stage Nitrifying RBC Systems' Operating Characteristics

Secondary Clarifier		
Surface Settling Rate (@ 8.6 m^3/d)	-	7.4 $m^3/d \cdot m^2$
Detention Time (@ 8.6 m^3/d)	-	1.7 hr
RBC		
Number of RBCs	-	5
Stages per RBC	-	2
Discs per Stage	-	9
Disc Diameter	-	0.5 m
Disc Area - Total	-	10.6 m^2
Rotational Speed	-	13 rpm
Peripheral Speed	-	0.34 m/sec
Hydraulic Loading[a]	-	81 $l/m^2 \cdot d$

[a]The hydraulic loading for all five RBC units was nominally 81 $l/m^2 \cdot d$ (2 $gal/d \cdot ft^2$), the exact hydraulic loading for each RBC is found in Table 6.3. The hydraulic loading calculation is based upon the assumption that each 2-stage RBC system contained the first two stages of a 4-stage RBC.

competition on the discs of the second stage. The control RBC and the alkaline chemical feed RBC systems were operating at approximately the same level of nitrification performance after a little more than three weeks of operation. The levels of performance for all five systems were very similar for about the next ten days. The overall performance of the control system, operating at the lower pH level, started to deteriorate after approximately 35 days of operation. This deterioration in

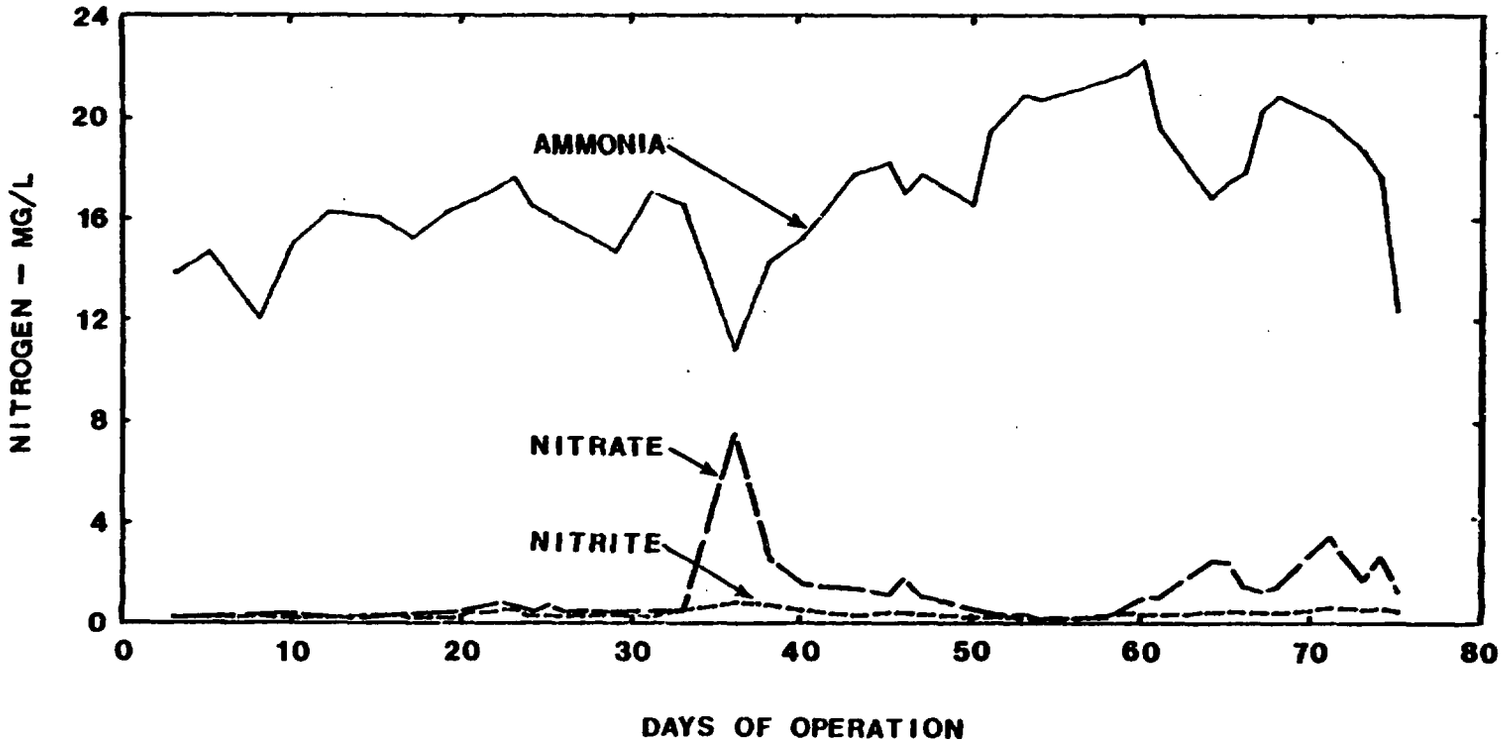


Figure 6.2 Influent Nitrogen Forms for the 2-Stage RBC Systems of the Alkaline Chemical Addition Study

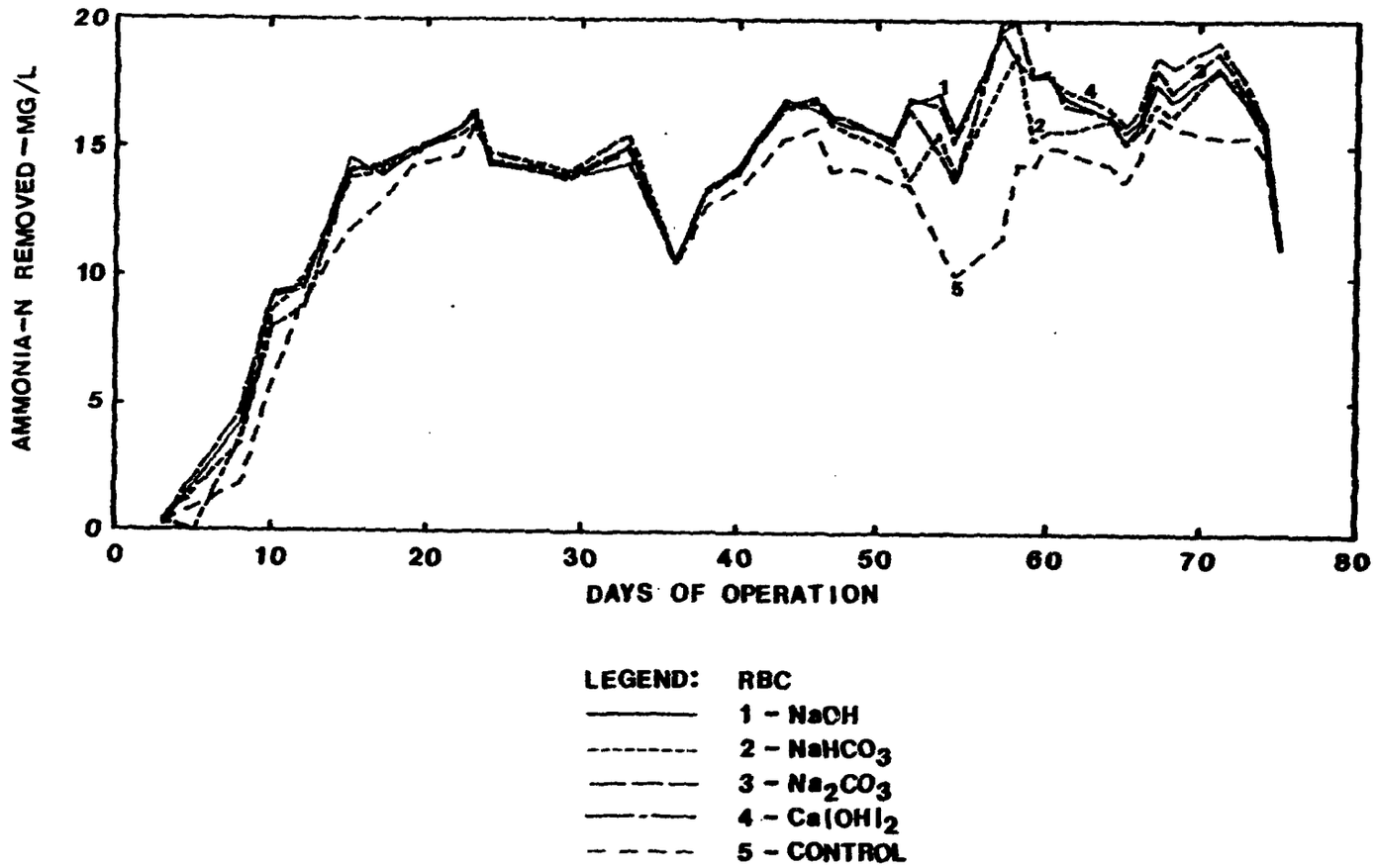


Figure 6.3 Relative Ammonia-Nitrogen Removals for the 2-Stage RBC Systems of the Alkaline Chemical Addition Study

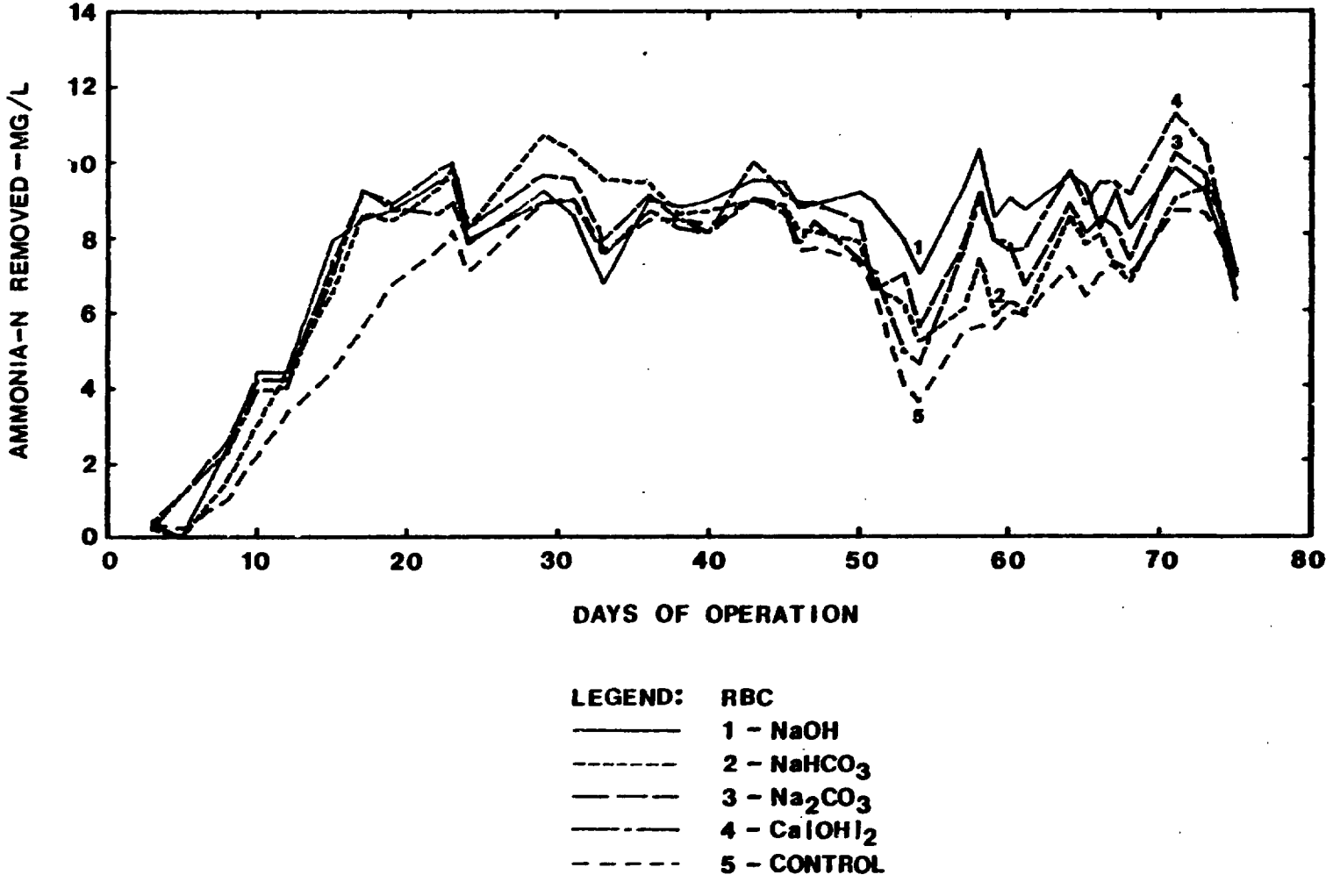


Figure 6.4 Relative Ammonia-Nitrogen Removals for the First Stages of the 2-Stage RBC Systems of the Alkaline Chemical Addition Study

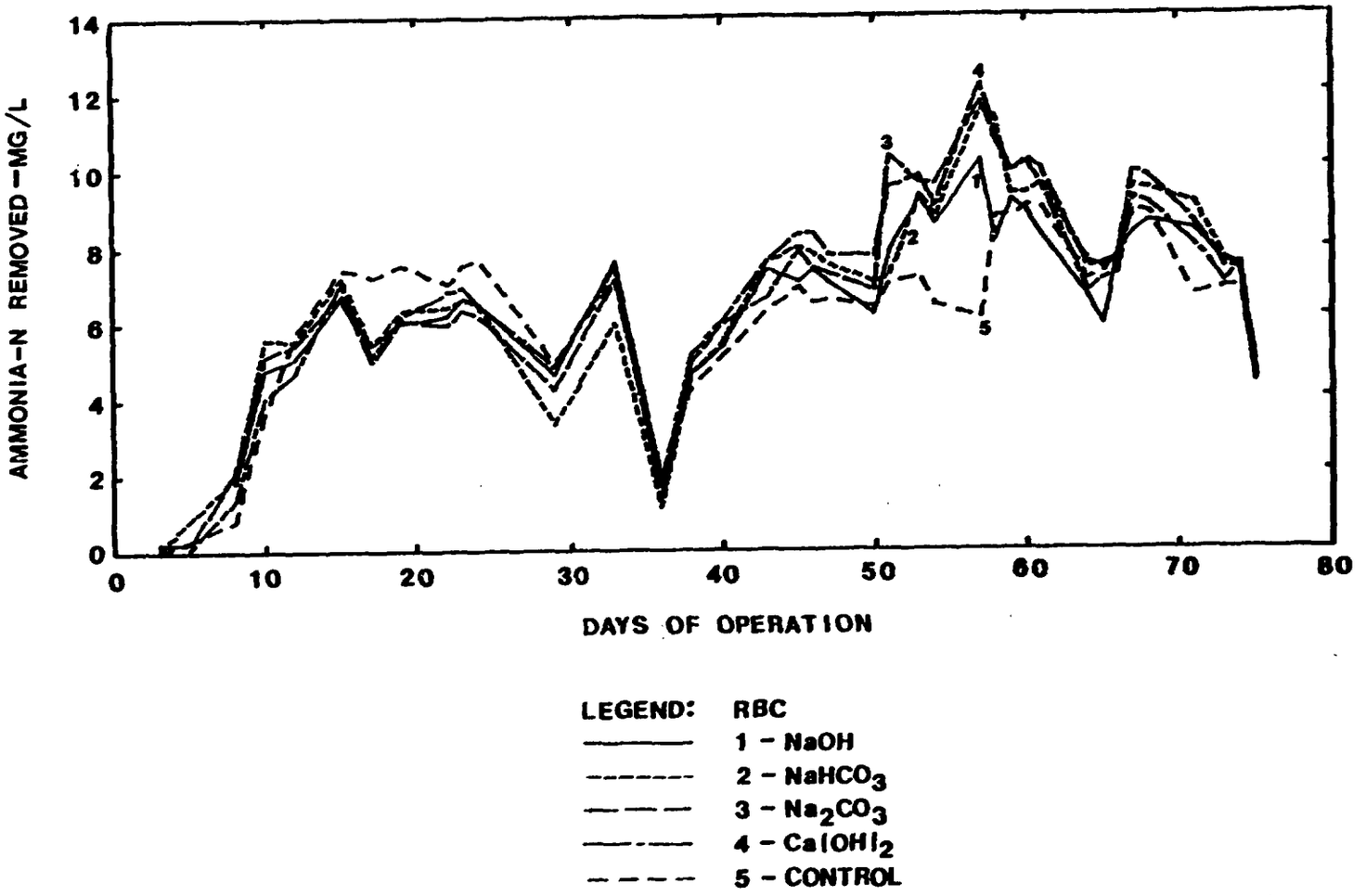


Figure 6.5 Relative Ammonia-Nitrogen Removals for the Second Stages of the 2-Stage RBC Systems of the Alkaline Chemical Addition Study

performance started initially in the second stage and was similar to that previously observed and reported in Sections 4.3.1 and 5.3.1.

Based upon the overall nitrification performance of the five 2-stage RBC systems, a period of relative dynamic equilibrium was established on approximately Day 38. A detailed comparison of the data from Day 38 to Day 75, for the RBC influent and stage 1 and stage 2 effluent wastewater characteristics for the five RBC systems, is presented in Tables 6.2, 6.3, and 6.4, respectively. The data on the relative amounts of total ammonia-nitrogen removed by the five RBCs during this period are presented in Table 6.5. These data show that the overall ammonia-nitrogen removals for the sodium hydroxide, sodium carbonate, and calcium hydroxide RBC systems were nearly the same. The performance level of the sodium bicarbonate RBC system was about 6 percent less that of the other three high pH systems. The control RBC, which was operated at the lowest pH conditions, removed about 16 percent less ammonia-nitrogen than did the three high pH alkaline chemical feed systems. Table 6.6 presents the ammonia removal data for each respective RBC stage. The data on the amounts of ammonia-nitrogen removed clearly demonstrate that the greatest removal occurs at the elevated pH conditions. The amount of ammonia removed by the first and second stages of the three high pH alkaline chemical feed RBC systems was essentially the same. The sodium bicarbonate RBC system had lower pH levels and lower performance in both stages. The control had the lowest stage pH levels and the poorest performance for both stages. Nitrogen balances during this period for all the stages of the five RBC systems are presented in Table 6.7. These nitrogen balances follow the same pattern previously reported in Sections 4.3.1 and 5.3.1. The percent recovery decreased slightly as pH

Table 6.2 RBC Influent Wastewater Characteristics for the Alkaline Chemical Addition Study[a]

Parameter	Mean	Std. Dev.
pH	6.5 (33)[b]	-
Alk ($CaCO_3$), mg/l	144 (24)	26
CBOD, mg/l	12.3 (18)	6.6
SS, mg/l	21 (17)	6
VSS, %	86 (17)	3
NH_3-N, mg/l	18.3 (24)	3.0
TKN-N, mg/l	22.8 (24)	3.5
Org-N, mg/l	4.6 (24)	2.1
(NO_2+NO_3)-N, mg/l	2.0 (24)	2.2
NO_2-N, mg/l	0.3 (24)	0.2
NO_3-N, mg/l	1.7 (24)	2.0
TP-P, mg/l	7.3 (3)	1.2
OP-P, mg/l	6.5 (3)	0.5
DO, mg/l	3.2 (14)	0.8
T, ^oC	20.5 (36)	0.8
Flow,[c] m^3/d	8.5 (30)	0.4

[a]Based upon data from Day 38 to Day 75.

[b]Number in parenthesis is the number of samples applied to statistical determinations.

[c]Flow is the total amount of wastewater going to all five RBC systems.

Table 6.3 RBC Stage 1 Effluent Wastewater Characteristics for the Alkaline Chemical Addition Study[a]

Parameter	RBC 1 (NaOH)		RBC 2 ($NaHCO_3$)		RBC 3 (Na_2CO_3)		RBC 4 ($Ca(OH)_2$)		RBC 5 (Control)	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
pH	8.5(34)[b]	-	7.5(34)	-	8.4(34)	-	8.5(34)	-	7.0(34)	-
Alk ($CaCO_3$), mg/l	251(26)	40	302(25)	77	415(25)	36	247(25)	36	94(25)	27
CBOD, mg/l	4.8(18)	1.2	4.7(18)	1.3	4.2(18)	1.5	3.8(18)	1.1	4.9(18)	1.6
SS, mg/l	32(18)	14	30(18)	13	35(18)	20	61(18)	25	24(21)	6
VSS, %	84(18)	2	83(18)	4	83(18)	3	54(18)	9	84(21)	4
NH_3-N, mg/l	10.3(26)	4.0	10.9(25)	3.8	10.2(25)	3.5	10.2(25)	3.6	11.5(25)	4.0
TKN-N, mg/l	14.5(26)	4.4	14.5(25)	4.3	13.9(25)	3.7	13.9(25)	3.7	15.0(25)	3.8
Org-N, mg/l	4.2(26)	2.1	3.5(25)	2.6	3.7(25)	2.3	3.7(25)	1.7	3.5(25)	1.6
(NO_2+NO_3)-N, mg/l	9.9(26)	3.3	9.6(24)	2.8	9.5(25)	3.5	9.8(25)	3.6	9.4(25)	3.1
NO_2-N, mg/l	1.1(26)	0.4	1.0(25)	0.2	1.0(25)	0.3	0.9(25)	0.4	0.7(25)	0.2
NO_3-N, mg/l	8.8(26)	3.3	8.7(24)	2.7	8.5(25)	3.4	8.8(25)	3.7	8.7(25)	3.0
TP-P, mg/l	7.2(3)	0.7	7.3(3)	1.1	6.8(3)	0.9	5.7(3)	0.7	7.3(3)	0.4
OP-P, mg/l	6.2(3)	0.2	6.4(3)	0.4	6.3(3)	0.3	4.5(3)	0.3	6.2(3)	0.2
DO, mg/l	4.3(15)	0.4	4.5(15)	0.4	4.4(15)	0.3	4.6(15)	0.3	4.7(15)	0.5
T, ^oC	20.4(37)	0.8	20.4(37)	0.8	20.4(37)	0.8	20.4(37)	0.8	20.4(37)	0.8
Flow, $l/m^2 \cdot d$	79(33)	4	79(33)	4	79(33)	4	79(33)	4	79(33)	5

[a]Based upon data from Day 38 to Day 75.

[b]Number in parenthesis is the number of samples applied to statistical determinations.

Table 6.4 RBC Stage 2 Effluent Wastewater Characteristics for the Alkaline Chemical Addition Study[a]

Parameter	RBC 1 (NaOH)		RBC 2 ($NaHCO_3$)		RBC 3 (Na_2CO_3)		RBC 4 ($Ca(OH)_2$)		RBC 5 (Control)	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
pH	7.9(34)[b]	-	7.7(34)	-	8.0(34)	-	7.9(34)	-	6.9(35)	-
Alk($CaCO_3$), mg/l	203(25)	28	242(25)	71	352(25)	35	191(25)	30	45(25)	21
CBOD, mg/l	3.3(18)	0.8	3.8(18)	1.2	3.0(18)	1.1	2.5(18)	0.6	3.9(18)	1.3
SS, mg/l	29(18)	9	29(18)	9	31(18)	13	48(18)	17	28(21)	9
VSS, %	85(18)	2	84(18)	2	83(18)	4	63(18)	5	86(20)	2
NH_3-N, mg/l	2.4(25)	1.5	3.1(25)	2.2	2.2(25)	1.5	2.2(25)	1.8	4.7(25)	3.1
TKN-N, mg/l	5.6(25)	2.1	6.2(25)	2.2	5.2(25)	1.8	5.2(25)	2.0	8.1(25)	3.3
Org-N, mg/l	3.2(25)	1.4	3.1(25)	1.2	3.0(25)	1.5	2.9(25)	1.4	3.5(25)	1.4
(NO_2+NO_3)-N, mg/l	17.2(24)	2.4	16.8(25)	2.5	17.1(25)	2.5	17.2(25)	2.7	15.7(25)	2.4
NO_2-N, mg/l	1.1(25)	0.6	1.5(25)	0.6	1.1(25)	0.7	1.0(25)	0.6	0.9(25)	0.3
NO_3-N, mg/l	16.1(24)	2.5	15.4(25)	2.7	15.9(25)	2.6	16.2(25)	2.8	14.8(25)	2.6
TP-P, mg/l	6.9(3)	0.6	7.0(3)	0.8	9.3(3)	3.4	6.3(3)	0.4	7.1(3)	0.4
OP-P, mg/l	6.3(3)	0.3	6.1(3)	0.6	6.3(3)	0.4	5.6(3)	0.4	6.4(3)	0.2
DO, mg/l	5.5(15)	0.7	5.7(15)	0.7	5.4(15)	0.8	5.3(15)	0.8	5.8(15)	0.6
T, °C	20.0(37)	0.7	20.0(37)	0.7	20.0(37)	0.7	20.0(37)	0.8	20.0(37)	0.8

[a]Based upon data from Day 38 to Day 75.

[b]Number in parenthesis is the number of samples applied to statistical determinations.

Table 6.5 Relative Rates of Nitrification for the RBC Systems of the Alkaline Chemical Addition Study[a]

RBC	Alkaline Chemical	Ammonia-N Removed (g NH_3-N/m^2·d)	Percent Removed	Percent of Maximum
1	NaOH	2.52	86	99
2	$NaHCO_3$	2.40	82	94
3	Na_2CO_3	2.54	87	100
4	$Ca(OH)_2$	2.55	87	100
5	Control	2.14	73	84

[a]Based upon data from Day 38 to Day 75.

Table 6.6 Relative Rates of Nitrification for the Stages of the RBC Systems of the Alkaline Chemical Addition Study[a]

RBC-Stage	Alkaline Chemical	pH	Ammonia-N Removed (g NH_3-N/m^2·d)	Percent[b] Removed	Percent of[c] Maximum
1-1	NaOH	8.5	2.53	43	98
2-1	$NaHCO_3$	7.5	2.33	40	91
3-1	Na_2CO_3	8.4	2.55	44	99
4-1	$Ca(OH)_2$	8.5	2.57	44	100
5-1	Control	7.0	2.14	37	83
1-2	-	7.9	2.50	77	98
2-2	-	7.7	2.46	72	97
3-2	-	8.0	2.52	78	99
4-2	-	7.9	2.54	78	100
5-2	-	6.9	2.14	59	84

[a]Based upon data from Day 38 to Day 75.
[b]Based upon ammonia-nitrogen influent to each RBC stage.
[c]Based upon maximum ammonia-nitrogen removed by calcium hydroxide RBC stages.

Table 6.7 RBC Nitrogen Balances for the Alkaline Chemical Addition Study[a]

RBC - Stage	Alkaline Chemical	pH	Total Nitrogen[b] mg/l	Percent Recovery	
				Stage[d]	RBC
INFLUENT	-	6.5	24.8(24)[c]	-	-
1-1	NaOH	8.5	24.4(26)	98	-
2-1	$NaHCO_3$	7.5	24.0(24)	97	-
3-1	Na_2CO_3	8.4	23.5(25)	95	-
4-1	$Ca(OH)_2$	8.5	23.7(25)	96	-
5-1	Control	7.0	24.4(25)	98	-
1-2	-	7.9	22.8(24)	93	92
2-2	-	7.7	23.0(25)	96	93
3-2	-	8.0	22.3(25)	95	90
4-2	-	7.9	22.3(25)	94	90
5-2	-	6.9	23.8(25)	98	96

[a]Based upon data from Day 38 to Day 75.

[b]Total nitrogen is total oxidized nitrogen plus total Kjeldahl nitrogen (TKN).

[c]Number in parenthesis is the number of samples utilized in the total nitrogen determinations.

[d]Nitrogen balances for the stages are based upon stage influent nitrogen.

increased. Again, this lower recovery is attributed to small ammonia losses due to ammonia stripping and denitrification within the thicker biofilms associated with the higher pH levels.

The influent CBOD data for all the RBC systems are presented graphically in Figure 6.6 and the CBOD removal data are summarized in Table 6.8. The level of CBOD decreased during the test except for the period

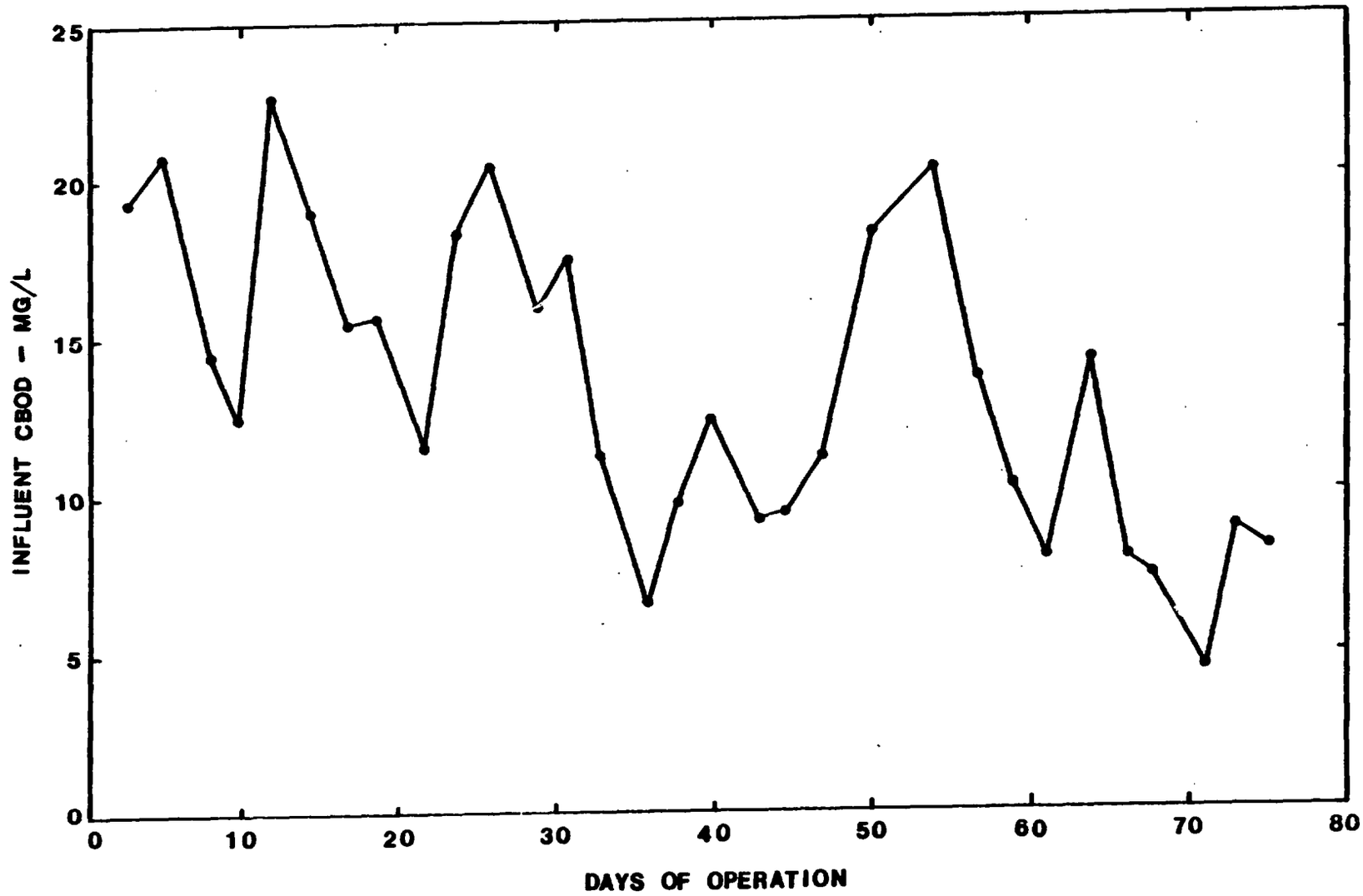


Figure 6.6 Influent CBOD for the 2-Stage RBC Systems of the Alkalinc Chemical Addition Study

Table 6.8 Relative CBOD Removals for the RBC Systems of the Alkaline Chemical Addition Study[a]

RBC-Stage	Alkaline Chemical	pH	CBOD-in mg/l	CBOD-out mg/l	CBOD Removed mg/l	Percent Removed Stage	Percent Removed Total
1 - 1	NaOH	8.5	12.3	4.8	7.5	61	-
1 - 2	-	7.9	4.8	3.3	1.5	31	73
2 - 1	$NaHCO_3$	7.5	12.3	4.7	7.6	62	-
2 - 2	-	7.7	4.7	3.8	0.9	19	69
3 - 1	Na_2CO_3	8.4	12.3	4.2	8.1	66	-
3 - 2	-	8.0	4.2	3.0	1.2	29	76
4 - 1	$Ca(OH)_2$	8.5	12.3	3.8	8.5	69	-
4 - 2	-	7.9	3.8	2.5	1.3	34	80
5 - 1	Control	7.0	12.3	4.9	7.4	60	-
5 - 2	-	6.9	4.9	3.9	1.0	20	68

[a]Based upon data from Tables 6.2, 6.3, and 6.4.

between Day 50 and Day 55. Return of supernatant from the anaerobic digestor into the trickling filter effluent caused a short term increase in the CBOD level. An examination of the data in Figures 6.3 through 6.5 reveals that the nitrification performance of the control RBC appeared to be affected more adversely by this transient CBOD condition than were the other higher pH RBC systems. The amount of ammonia removed by the first RBC stages decreased during this period due to the influx of CBOD; however, the amount of ammonia removed in the second RBC stages increased rapidly. The data in Table 6.8 reveal that much more CBOD (7.4 to 8.5 mg/l) was removed in the first RBC stages than in the second RBC stages (0.9 to 1.5 mg/l) of all the RBC systems.

The overall amounts of CBOD removed were greatest for the higher pH RBC systems. This observation provides further evidence that heterotrophic activity also was enhanced with increasing pH.

The data in Table 6.4 on the amount of suspended solids in the effluent from the second RBC stages reveal that the addition of sodium hydroxide, sodium bicarbonate, and sodium carbonate caused only a slight increase of from 1 to 3 mg/l in the suspended solids in the RBC effluents; whereas the use of calcium hydroxide increased the suspended solids by 20 mg/l. These suspended particulates would not redissolve after mixing for 1 hour. The observed increase in solids was attributed to the reaction between the calcium hydroxide and the carbonic acid or carbon dioxide in the wastewater to form calcium carbonate.

6.3.2 Biofilm Development and Microbial Enumerations

The RBC systems had no biofilm initially, however a biofilm which could be sensed by touch had developed on the discs of all stages within 36 hours. Within 72 hours from startup, all first stage discs had developed biofilms which were noticeably heavier than the second stage biofilms. The characteristic tan color associated with nitrifying biofilms had developed by Day 4 and was more apparent in the first stage biofilms. All of the biofilms were very uniform in texture. This initial biofilm growth appeared to be heavier than the biofilms developed during the previous research phases. By Day 6, the trough walls also had developed noticeable amounts of biofilm. Although both stage biofilms got heavier and darker with time, the heavier and darker biofilms were on the first stage discs. The first stage biofilms became brown while those on the second stage discs remained tan to bronze in color. By Day 16, discs in all the first stages had experienced some sloughing

while those in the second stages retained their uniformity. The loss of uniformity in the second stages commenced about Day 21. As time progressed, the loss of biofilm uniformity was greatest for the control and the sodium bicarbonate RBC systems. The first stages of the calcium hydroxide, sodium carbonate, and the sodium hydroxide RBC systems had the heaviest and most uniform biofilm coatings. The biofilm uniformity related directly to the ammonia removal performance levels of the RBC systems (see Figures B.3-B.6, Appendix B). The loss of biofilm during the 75 day period was attributed mainly to hydraulic shear starting on the surface of the biofilm and progressing inward. Biofilm sloughing from the bare disc outward did not occur continuously. The former method of sloughing appeared to be associated with relatively low and steady CBOD loadings, while the latter form of sloughing appeared to be associated with relatively high and fluctuating CBOD loadings.

Snails in the troughs were noticed first on Day 21. The snails would not inhabit troughs in which the wastewater was maintained at pH 8.5. The highest population of snails was noted in the pH 7.5 first stage of the sodium bicarbonate RBC system. Lesser numbers of snails were noted in the lower pH stages of the control RBC system. The snails had entered the second stages of all RBC systems by the end of the study period.

The data for the development of total biofilm for all the stages of the five RBC systems, as well as for the trough walls of the control RBC, are presented in Figures 6.7 and 6.8. These data show that the biofilms on the first stage discs of all five RBC systems developed at a greater rate than previously experienced. This increased rate was

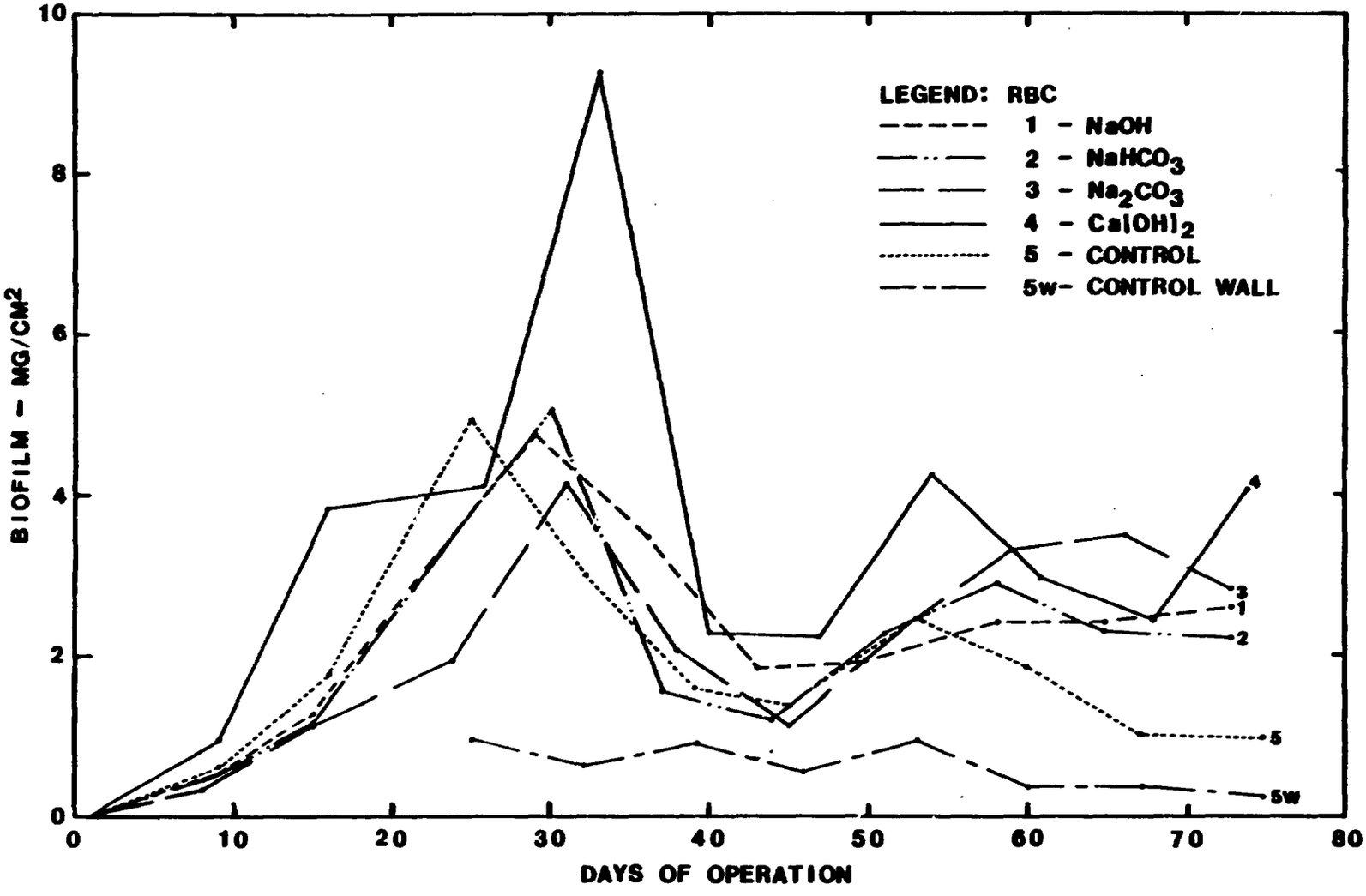


Figure 6.7 First Stage Disc Biofilm Development in the RBC Systems of the Alkaline Chemical Addition Study

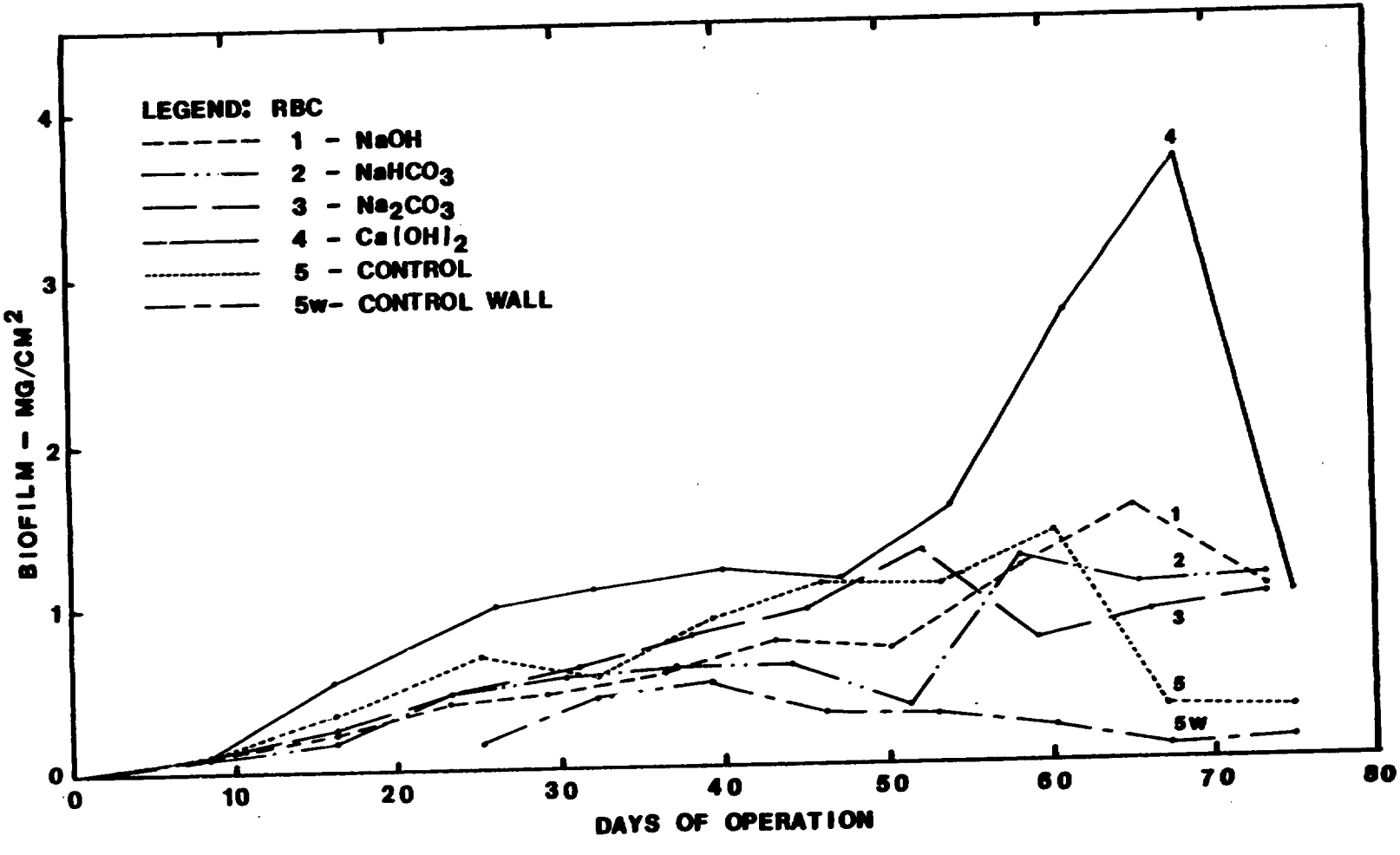


Figure 6.8 Second Stage Disc Biofilm Development in the RBC Systems of the Alkaline Chemical Addition Study

the result of having the startup date (Day 1) coincide with the first day of the PSU fall term. The sudden influx of students resulted in increased levels of CBOD in the PSU WWTP high rate trickling filter effluent. This increased CBOD loading resulted in enhanced heterotrophic activity on all discs; however, it was not high enough to impede the development of populations of nitrifying bacteria. Within two to three weeks, the RBC systems had achieved their maximum rates of ammonia oxidation. After three weeks of operation, the biofilm concentrations were 2.5, 1.6, 3.9, 2.5, and 3.3 mg/cm^2 for the first stages of the sodium hydroxide, sodium carbonate, calcium hydroxide, sodium bicarbonate, and the control RBC systems, respectively. The first stages of all five RBC systems underwent severe sloughing after approximately five weeks of operation. A second period of enhanced heterotrophic activity occurred from Day 50 to Day 55. This higher activity was due to a temporary increase in CBOD as discussed in Section 6.3.1 and is described in Figure 6.6. This change resulted in a sharp increase in first stage biofilm development, a displacement of some of the nitrifying population from the active biofilm layers, and a temporary drop in overall nitrification efficiency of the first stages as noted in Section 6.3.1 above.

The variations in biofilm concentrations, as shown in Figures 6.7 and 6.8, were greatest on the first stages. The growths on the trough walls of both stages of the control RBC system were much less than on the respective discs. This higher growth on the discs is attributed to an improved substrate availability on the disc surfaces due to greater turbulence and an enhanced dissolved oxygen transfer potential associated with periodic exposure of the discs to the atmosphere (57). The

data for the RBC biofilm concentrations and percent volatile matter are presented in Table 6.9. These data show that the highest biofilm concentrations are associated with the higher pH levels. Only the addition of calcium hydroxide resulted in an increase in inert matter entrained within both the first and second stage biofilms. This observation is in agreement with the suspended solids data in Section 6.3.1 which show that only the calcium hydroxide RBC system experienced an overall increase in suspended solids and inert content. Sodium hydroxide, sodium carbonate, and sodium bicarbonate additions did not affect the biofilm volatile content; however, a slight increase in volatile content in all the second stage biofilms was noted. When the inert matter data are removed from the biofilm data presented in Figures 6.7 and 6.8, the volatile content of the biofilms of the calcium hydroxide RBC system are about the same as the volatile content of those biofilms of the other RBCs.

Table 6.10 contains a data summary of the amount of nitrogen and selected metals contained within each biofilm. The percent nitrogen within the films was between 5 and 8 percent, as compared to the 8 to 9 percent for RBC biofilms as reported by Ouyang (77). The amount of calcium within each of the biofilms was approximately 2 percent, except for the calcium hydroxide RBC biofilm which had 12 and 9.6 percent in the biofilms of the first and second stage, respectively. The additional calcium within the calcium hydroxide RBC biofilms was assumed to be mostly in the form of calcium carbonate. The data on the amount of metals within the wastewaters of the various RBC systems are presented in Table 6.11. These data basically reflect sodium and calcium changes

Table 6.9 Mean Biofilm Concentrations and Percent Volatile Matter in the RBC Disc Biofilm of the Alkaline Chemical Addition Study[a]

RBC - Stage	Alkaline Chemical	Stage pH	Biofilm mg/cm^2	Volatile Biofilm %
1-1	NaOH	8.5	2.45	86
2-1	$NaHCO_3$	7.5	2.08	86
3-1	Na_2CO_3	8.4	2.51	87
4-1	$Ca(OH)_2$	8.5	3.03	66
5-1	Control	7.0	1.57	87
1-2	--	7.9	0.97	89
2-2	--	7.7	0.84	90
3-2	--	8.0	0.96	89
4-2	--	7.9	1.88	70
5-2	--	6.9	0.87	90

[a]Samples taken at weekly intervals from Day 36 to Day 75.

resulting from the chemical addition. The levels of sodium observed were well below the levels which might have created an inhibitory effect (78).

The populations of ammonia-oxidizing, nitrite-oxidizing, and heterotrophic bacteria were monitored for both stages of each RBC system. Figures 6.9 and 6.10 present graphically the data on the relative geometric mean bacteria populations per unit of disc area and per unit weight of dry volatile biofilm for the stages of each RBC system from Day 36 to Day 75. This time period corresponds to the same period over which the relative nitrification rates are compared in Section 6.3.1.

Table 6.10 Percent Nitrogen and Metals Contained Within the RBC Disc Biofilms of the Alkaline Chemical Addition Study[a]

RBC - Stage	Alkaline Chemical	Percent[b] Nitrogen	Metals Content - Percent[c] Ca^{++}	Mg^{++}	Na^{+}
1-1	NaOH	5.7	2.0	0.5	1.1
2-1	$NaHCO_3$	5.7	1.9	0.5	1.3
3-1	Na_2CO_3	7.5	2.5	0.5	1.5
4-1	$Ca(OH)_2$	7.0	12.	0.6	0.6
5-1	Control	7.6	1.7	0.5	1.2
1-2	—	5.4	1.9	0.5	2.2
2-2	—	7.6	2.3	0.7	3.0
3-2	—	6.4	1.9	0.5	2.6
4-2	—	6.0	9.6	0.5	1.2
5-2	—	6.8	2.6	1.4	5.5

[a]Based Upon eight sets of samples taken at weekly intervals from Day 23 to Day 75.

[b]Percent nitrogen is based upon volatile solids.

[c]Percent metal is based upon total solids.

Table 6.11 Metals Content of the Wastewater Within the Stages of the RBC Systems of the Alkaline Chemical Addition Study[a]

RBC - Stage	Alkaline Chemical	Metals Content - mg/l Ca^{++}	Mg^{++}	Na^{+}	K^{+}
Influent	--	37	20	60	15
1 - 1	NaOH	37	20	136	15
2 - 1	$NaHCO_3$	38	20	157	16
3 - 1	Na_2CO_3	38	21	209	16
4 - 1	$Ca(OH)_2$	103	21	62	15
5 - 1	Control	37	20	62	15
1 - 2	--	37	20	133	15
2 - 2	--	37	20	152	16
3 - 2	--	39	20	220	16
4 - 2	--	103	20	61	14
5 - 2	--	38	20	61	14

[a]The mean percentages presented are based upon five sets of samples for each location taken between Day 45 and Day 73.

The ratios of the populations for the three groups of bacteria for the stages of each RBC system are presented in Table 6.12. The populations of all three bacteria per unit area were greater for the first stages of the three high pH, high performance systems than for the first stages of sodium bicarbonate and control RBC systems. The first stages of the former were maintained at pH 8.4 to pH 8.5 while the latter were maintained at pH 7.5 and 7.0 for the sodium bicarbonate and control RBC systems, respectively. In the second stages, where there was less CBOD, less disc biofilm, and no pH control, the population differences were not as dramatic. The ratios in Table 6.12 reveal that the

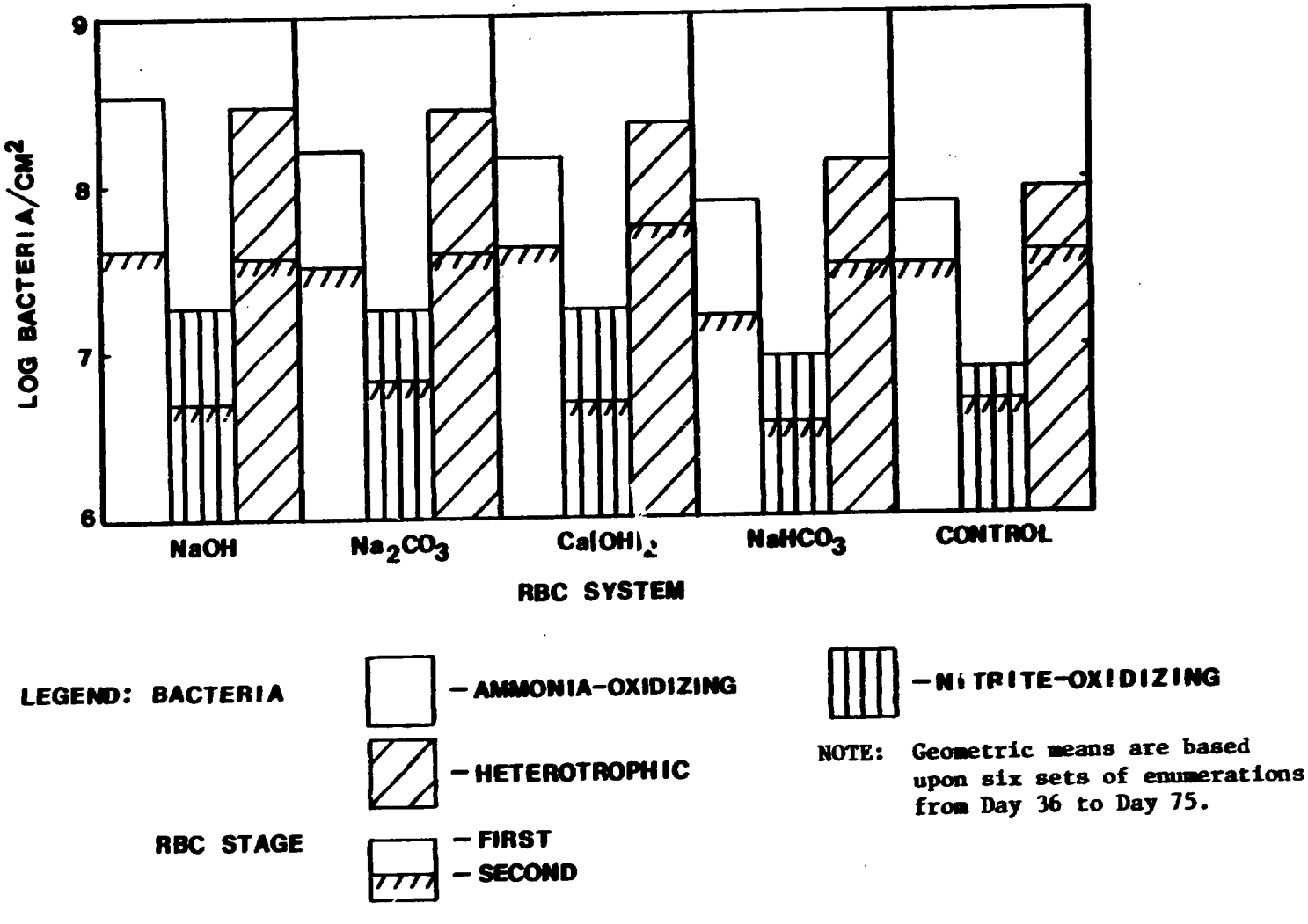


Figure 6.9 Relative Geometric Mean Populations of Ammonia-Oxidizing, Nitrite-Oxidizing, and Heterotrophic Bacteria on a Unit Disc Area Basis for the Stages of the RBC Systems of the Alkaline Chemical Addition Study

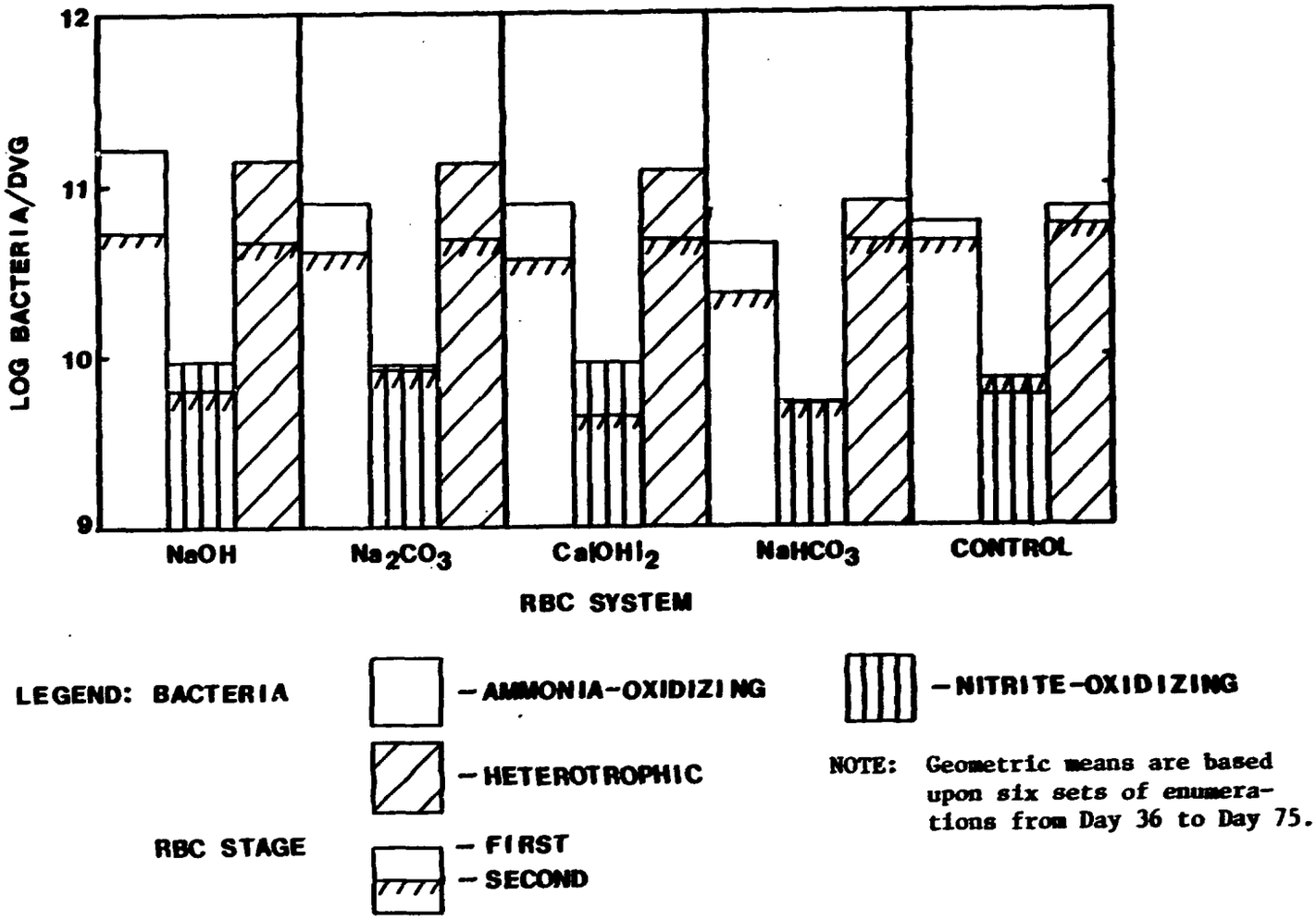


Figure 6.10 Relative Geometric Mean Populations of Ammonia-Oxidizing, Nitrite-Oxidizing, and Heterotrophic Bacteria on a Unit Weight of Dry Volatile Biofilm Basis for the Stages of the RBC Systems of the Alkaline Chemical Addition Study

populations of heterotrophic and ammonia-oxidizing bacteria, relative to nitrite-oxidizing bacteria, decreased when progressing from stage one to stage two.

Table 6.12. Ratio of Heterotrophic:Ammonia-Oxidizing:Nitrite-Oxidizing Bacteria for the RBC Stages of the Alkaline Chemical Addition Study[a]

RBC	Heterotrophs:Ammonia-Oxidizers:Nitrite-Oxidizers	
	Stage 1	Stage 2
NaOH	14 : 16 : 1	7.1 : 8.0 : 1
Na_2CO_3	14 : 8.6 : 1	5.5 : 4.7 : 1
$Ca(OH)_2$	12 : 7.8 : 1	11. : 7.9 : 1
$NaHCO_3$	13 : 7.5 : 1	8.1 : 4.0 : 1
Control	11 : 9.4 : 1	7.3 : 6.1 : 1

[a]Ratios are based upon the geometric mean of 6 sets of samples taken at weekly intervals from Day 36 to Day 75.

A comparison of the activity levels for the ammonia-oxidizing and nitrite-oxidizing bacteria is presented in Table 6.13. These data demonstrate that lower levels of activity were associated with the higher performance RBC systems and that the level of activity was greater in the second stages than in the first stages. These findings are contradictory to the expectation that higher bacterial performance will be associated with the high pH RBC systems and that bacterial performance in the first stage will be greater than in latter stages. These seemingly contradictory findings probably are due to the fact the enumeration procedures include bacteria within the active biofilm layer as well as inactive bacteria existing within the biofilm below the active layer. Those bacteria below the active layer normally would not

be expected to engage extensively in the nitrification process but are still capable of demonstrating viability. The maximum potential activity shown in the data in Table 6.13 reflect the potential nitrification capacity of the RBC systems if all the nitrifying bacteria identified could be induced to function at their respective maximum activity levels. Maximum potential activity levels less than 100 percent reflect higher bacterial populations present than were identified in the enumeration procedure.

Table 6.13 Actual and Maximum Potential Activity Levels for the Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria on the Discs of the RBCs of the Chemical Addition Study

RBC - Stage	Alkaline Chemical	Oxidizing Activity[a] Ammonia pmole/cell·hr	Oxidizing Activity[a] Nitrite pmole/cell·hr	Max. Potential Activity[b] Ammonia %	Max. Potential Activity[b] Nitrite %
1-1	NaOH	0.0023	0.037	870	27
2-1	$NaHCO_3$	0.0094	0.074	210	14
3-1	Na_2CO_3	0.0052	0.041	390	24
4-1	$Ca(OH)_2$	0.0054	0.041	370	24
5-1	Control	0.0087	0.084	230	12
1-2	--	0.018	0.15	110	7
2-2	--	0.046	0.19	43	5
3-2	--	0.023	0.11	87	9
4-2	--	0.018	0.15	110	7
5-2	--	0.019	0.12	110	8

[a] Based upon data from Tables 6.2, 6.3, 6.4, and Figure 6.9.

[b] The maximum potential activity levels (MPN times maximum activity levels per bacteria) are expressed as a percent of the observed activity levels. The maximum potential activity levels are based upon maximum bacteria activity levels reported by Belser (13) of 0.02 and 0.01, pmole/cell·hr for ammonia-oxidizing and nitrite-oxidizing bacteria, respectively.

6.3.3 RBC Microbial Distribution

A substudy was conducted on the two stages of the control RBC system to examine the distribution of the three bacteria populations among the RBC discs, the RBC trough walls, and the wastewater within the two RBC stages. The RBC trough walls contain about 9 percent of the total RBC surface area. The data from this substudy are presented in Table 6.14. The data show that between 95 and 98 percent of the heterotrophic and nitrifying bacteria resided within the biofilm on the RBC discs. The RBC trough walls had only 1 to 2 percent of the bacteria and the trough wastewater had from 1 to 3 percent. Based upon the findings of Section 6.3.2, regarding levels of bacterial activity, it would be inappropriate to assume that the true nitrification capacity within any particular stage is directly proportional to the relative bacteria populations on the disc, walls, and in the trough wastewater. However, these data do support the concept that the RBC disc area provides the major microbial base for the nitrification process.

6.3.4 pH Effect on Heterotrophic Activity and Biofilm Development

Results of this research effort had indicated that heterotrophic activity and biofilm development were enhanced under elevated pH conditions (Sections 4.3.2, 5.3.2, and 6.3.2). In order to provide additional information regarding this observation, approximately 400 cm^2 of new disc material was added to the first stage discs of the control (pH 7.0), the sodium bicarbonate (pH 7.5), and the sodium hydroxide (pH 8.5) RBC systems on Day 62. The development of biofilm and the establishment of heterotrophic populations on these discs were monitored through Day 77. The resulting data are presented in Figures 6.11 and 6.12. The data demonstrated that both the biofilm and the

Table 6.14 Distribution of Ammonia-Oxidizing, Nitrite-Oxidizing, and Heterotrophic Bacteria Within a Nitrifying 2-Stage RBC.

RBC - Stage	Location	Total Bacteria (%)[a] Ammonia-Oxidizing Bacteria	Nitrite-Oxidizing Bacteria	Heterotrophic Bacteria
5[b] - 1	Disc	3.9×10^{12} (97)	3.9×10^{11} (98)	4.8×10^{12} (97)
	Wall	9.3×10^{10} (2)	3.6×10^{9} (1)	8.9×10^{10} (2)
	Trough	1.7×10^{10} (1)	4.9×10^{9} (1)	7.7×10^{10} (1)
5 - 2	Disc	1.7×10^{12} (96)	2.5×10^{11} (98)	2.0×10^{12} (95)
	Wall	4.1×10^{10} (2)	2.9×10^{9} (1)	4.3×10^{10} (2)
	Trough	2.9×10^{10} (2)	3.5×10^{9} (1)	5.6×10^{10} (3)

[a]Number in parenthesis is the percentage of the total number of bacteria. Data are based upon six sets of data for each location from Day 32 to Day 75.

[b]RBC 5 was the control RBC during the alkaline chemical addition study.

heterotrophic activity developed more rapidly as pH increased from pH 7.0 to pH 8.5. During this 15-day test period, the influent CBOD concentration was approximately 8 mg/l. These same three RBC stages demonstrated much more similar biofilm growth rates (Figure 6.7) during the first 30 days of the chemical addition study. However, during this earlier 30-day period, the CBOD level was approximately 17 mg/l, or more than double the amount of CBOD observed during this latter study. These results indicate that the ability of elevated pH levels to enhance heterotrophic activity and biofilm development may be greatest during the relatively low CBOD loadings normally associated with nitrification. Significant increases in CBOD may overshadow the more subtle

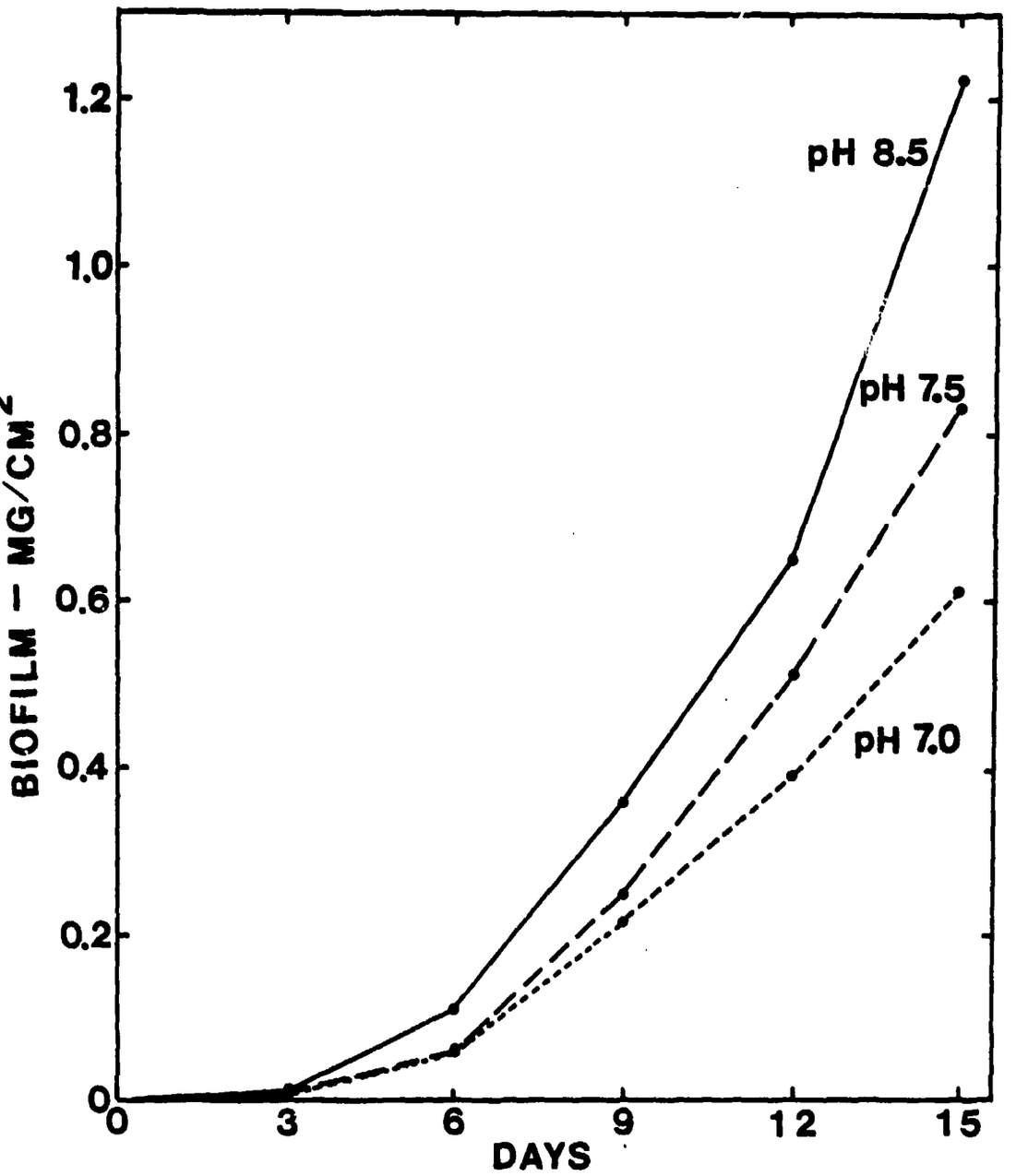


Figure 6.11 Relative RBC Biofilm Development under pH Conditions from pH 7.0 to pH 8.5

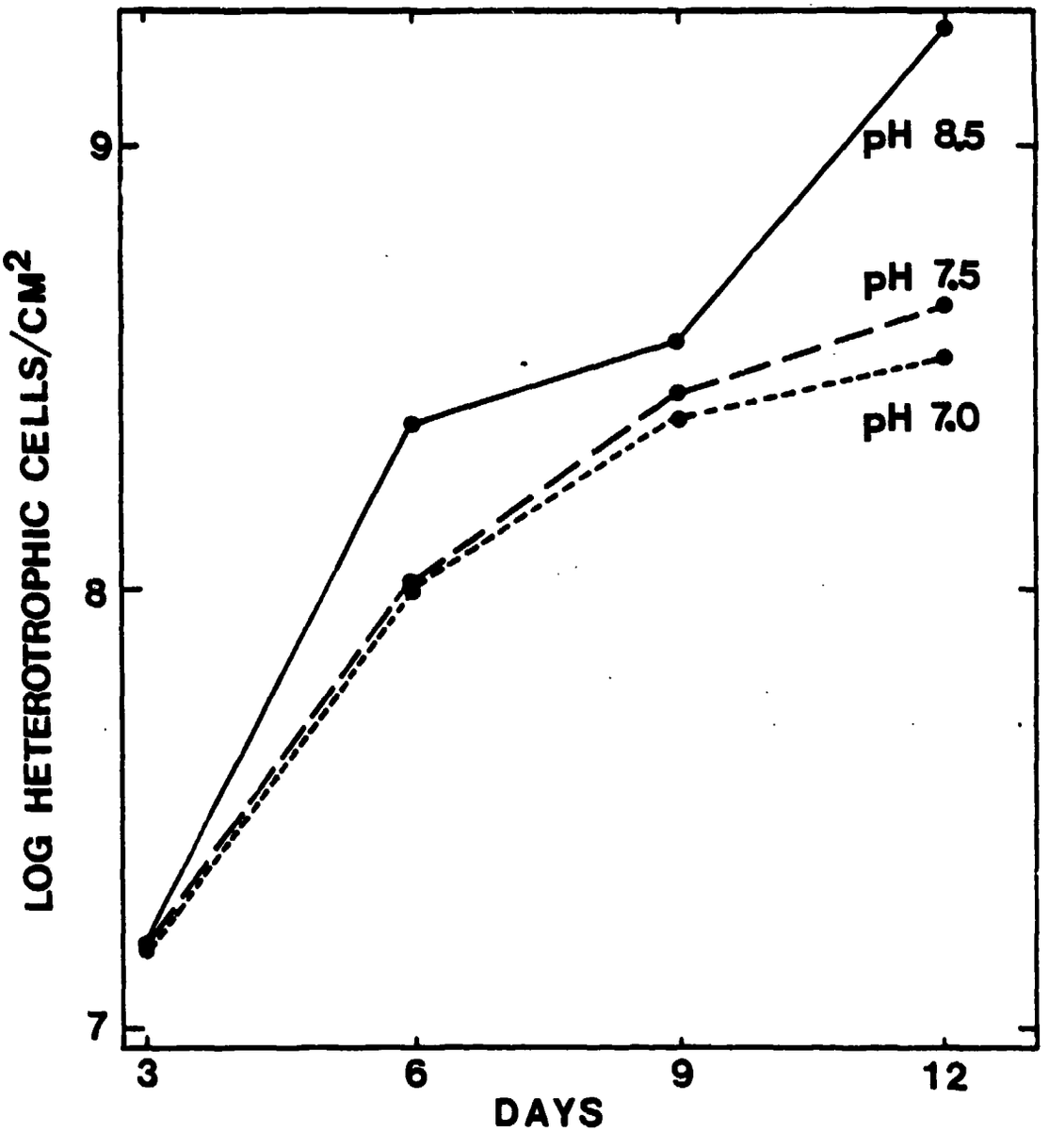


Figure 6.12 Relative RBC Heterotrophic Bacteria Growth Under pH Conditions from pH 7.0 to pH 8.5

influence of pH. Heterotrophic activity within the nitrifying RBC is discussed in greater detail by Doherty (26).

6.3.5 Alkalinity Destruction During Nitrification

The data for four simultaneous alkalinity destruction tests conducted on Day 94 on the first stages of the sodium hydroxide, sodium carbonate, calcium hydroxide, and control RBCs are presented in Figure 6.13. During these tests, the ammonia-nitrogen level of each RBC trough was increased by the addition of ammonium chloride, and then the flow into each RBC stage was stopped temporarily. Each RBC stage was treated as a batch system and changes in alkalinity and residual ammonia were monitored. The slopes' reciprocals in Figure 6.13 represent alkalinity destroyed per ammonia-nitrogen oxidized. The similar slope of the sodium hydroxide, sodium carbonate, and control RBC systems reveal similar alkalinity destruction values. However, the calcium hydroxide RBC system had a much steeper slope, indicating a much lower alkalinity destruction rate. This steeper slope was the result of either a significant change in the bacterial response to calcium hydroxide, or neutralization within the biofilm itself. This latter theory was tested by evaluating the acid neutralization capacity (see Appendix A) of each biofilm. The resulting data are presented in Table 6.15. The data clearly show that the calcium hydroxide biofilm possessed a vastly enhanced neutralization capacity beyond that possessed by the other biofilms. These data support the theory that an enhanced neutralization capacity is contained in the biofilm which reduces the bulk solution neutralization requirements.

A second set of simultaneous alkalinity destruction tests was conducted on Day 101 using the first stages of the control and the sodium

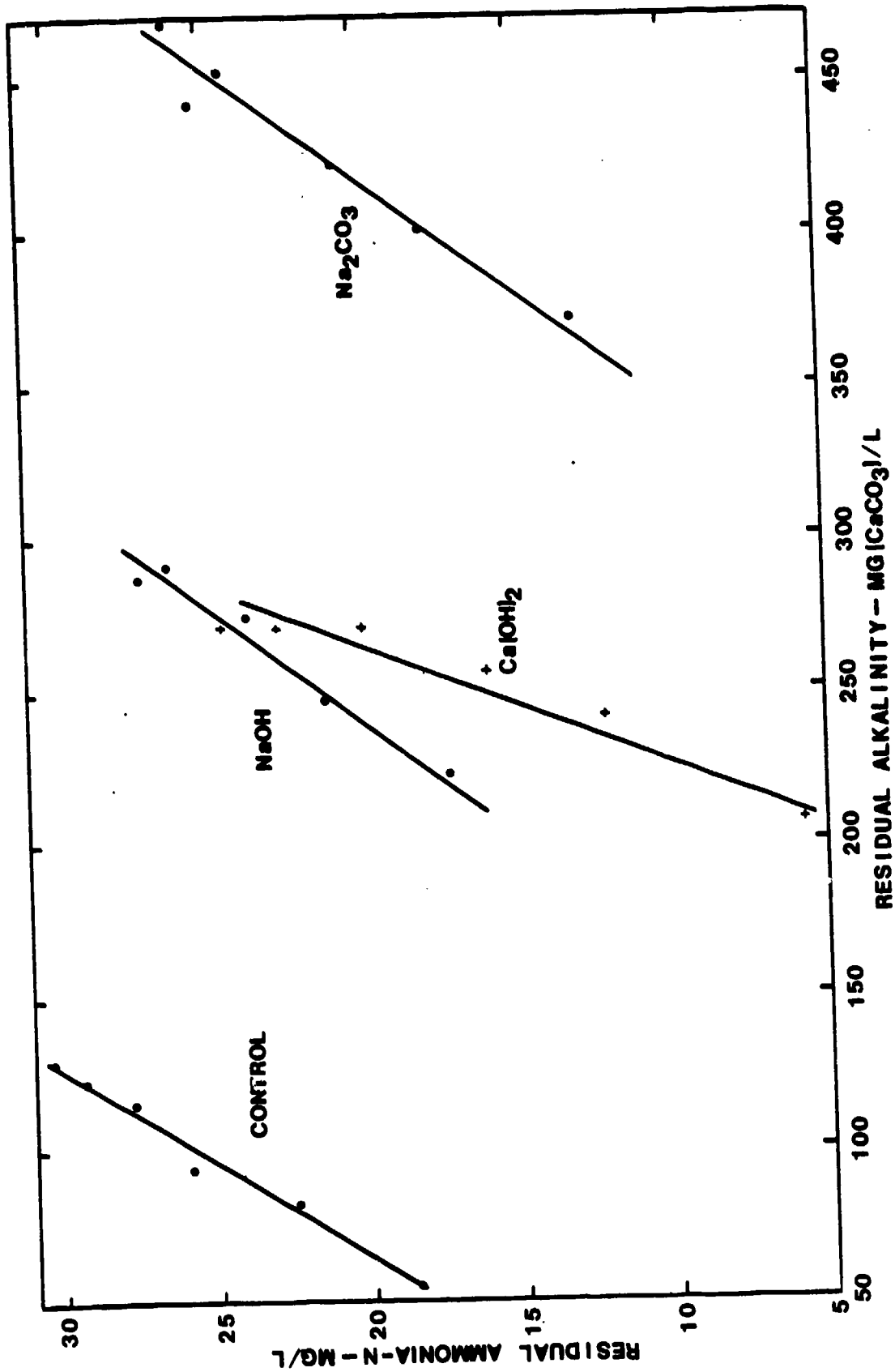


Figure 6.13 Batch Alkalinity Destruction Tests During the Alkaline Chemical Addition Study

Table 6.15 Acid Neutralization Capacity of RBC Biofilms Developed During the Alkaline Chemical Addition Study[a]

RBC - Stage	Alkaline Chemical	Biofilm Alkalinity[b] $mg(CaCO_3)/cm^2$
1-1	NaOH	0.23
3-1	Na_2CO_3	0.25
4-1	$Ca(OH)_2$	1.86
5-1	Control	0.04
1-2	--	0.08
3-2	--	0.13
4-2	--	0.68
5-2	--	0.03

[a]Based upon a single 50 cm^2 biofilm sample from each RBC disc on Day 99. Stage 2-1 and 2-2 biofilms were not analyzed because they were undergoing reversion at the time of the sampling.

[b]All biofilms initially manifested a slightly basic character except for the control biofilms which were slightly acidic.

bicarbonate RBCs. The latter RBC had been without alkaline chemical addition since Day 75 and undergoing reversion. It had nitrifying characteristics similar to the 5-1 RBC stage. Ammonium chloride was added to both systems, except that the ammonia concentration in the 2-1 RBC stage was made approximately 33 percent greater than that in the 5-1 RBC stage. The results of the batch system tests are presented in Figure 6.14. These tests reveal that the ammonia removal was relatively constant for both systems for the initial 80 minutes of the test. As expected, the pH was depressed in both systems as the ammonia was oxidized. At approximately 80 minutes into the test, the rate of nitrification within both systems started to decrease. At

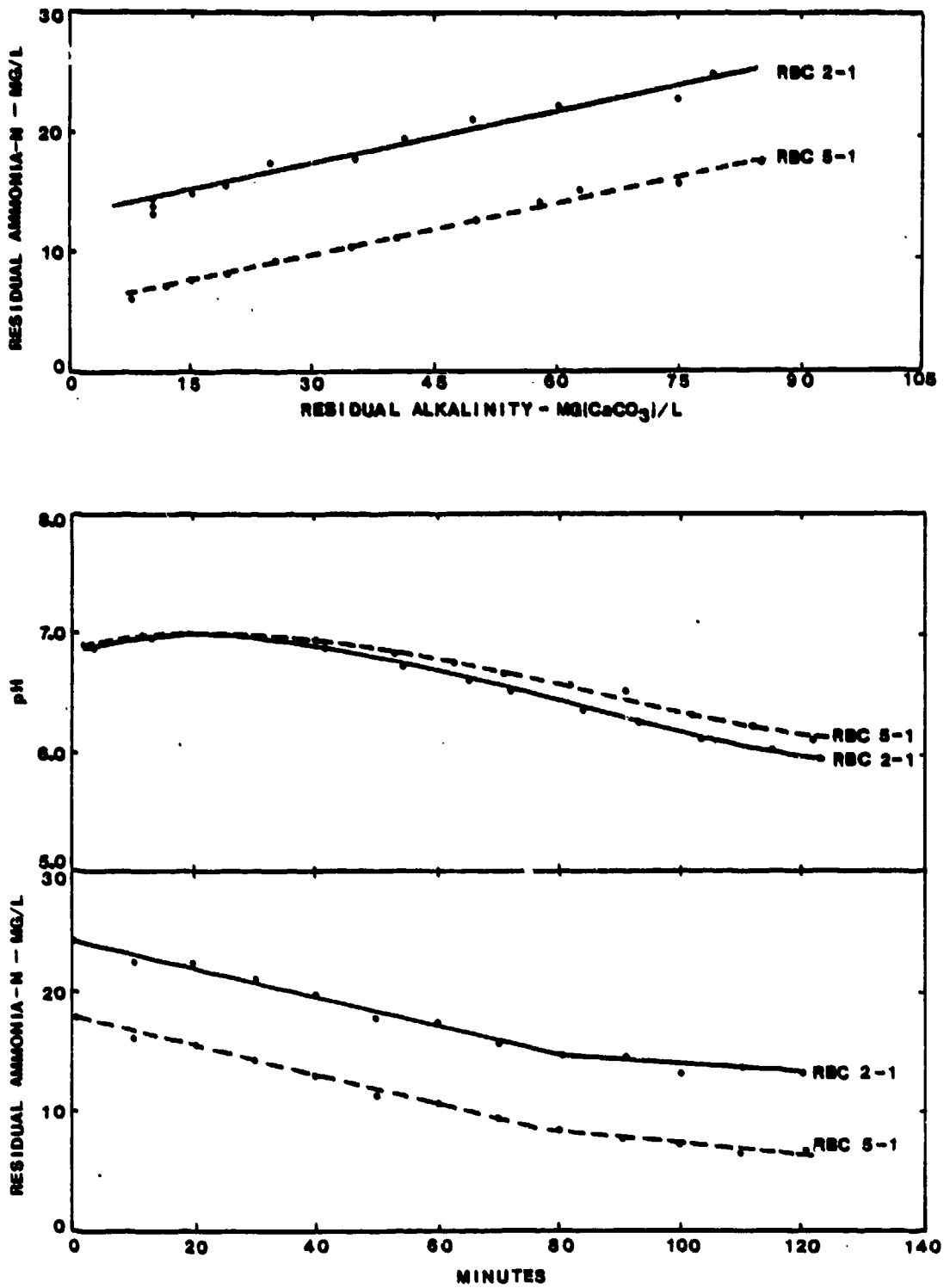


Figure 6.14 Batch Alkalinity Destruction Tests for Similar RBC Systems Under Varying Ammonia and Alkalinity Conditions

this time, both systems had a pH of approximately pH 6.5; however, the ammonia concentrations and alkalinity levels were different. This observation provides further evidence that the pH is the primary factor affecting nitrification rather than alkalinity per se. The alkalinity destruction curves shown in Figure 6.14 reveal that the level of alkalinity destruction is relatively constant even down to bulk solution alkalinity concentrations as low as 7.5 mg $CaCO_3$/l. The alkalinity destruction data for this research phase are summarized in Table 6.16.

6.3.6 2-Stage Alkaline Chemical Addition

On Day 75, the sodium carbonate RBC system was modified in order to add sodium carbonate to the second stage as well as to the first stage. Both stages were maintained at approximately pH 8.5. The performance of this 2-stage alkaline chemical feed RBC system was compared against the calcium hydroxide RBC system having only single stage pH control in the first stage. This phase of the research was terminated after 28 days on Day 103 with the start of the PSU Christmas break. It was recognized at the onset of this substudy phase that insufficient time remained before the PSU Christmas break to gather adequate long term data upon which to base definitive conclusions, particularly in view of the length of time already observed as necessary to establish changes in performance levels. However, dramatic effects might still be observed and trends might be indicated which could form the basis for future research.

Comparative ammonia removal data for this period are presented in Figure 6.15. Based upon these short term data, 2-stage pH control did not demonstrate improved nitrification over single-stage pH control.

The appearance of the second stage biofilm of the sodium carbonate RBC system did not demonstrate any obvious improvement in uniformity or disc coverage.

Table 6.16 RBC Nitrification and Alkalinity Destruction During the Alkaline Chemical Addition Study

RBC - Stage	Alkaline Chemical	Alkalinity Destruction (mg $CaCO_3$/mg NH_3-N) Continuous Operation[a]	Batch Operation[b]
1-1	NaOH	--	6.2
2-1	$NaHCO_3$	--	6.8
3-1	Na_2CO_3	--	7.4
4-1	$Ca(OH)_2$	--	3.8
5-1	Control	7.4	6.6
1-2	--	6.1	--
2-2	--	7.7	--
3-2	--	7.9	--
4-2	--	7.0	--
5-2	--	7.2	--

[a]Based upon data presented in Tables 6.2, 6.3, and 6.4.
[b]Based upon data presented in Figures 6.13 and 6.14.

6.3.7 RBC Hydraulic Tracer Study

A hydraulic tracer study, similar to the study described in Section 5.3.6, was conducted on Day 105 in order to examine if the hydraulic characteristics of the 2-stage RBC system operating with extenders differed significantly from those of the same system without extenders. Two hydraulic tracer studies were conducted on the same RBC system. The first test was conducted with the extenders in place and

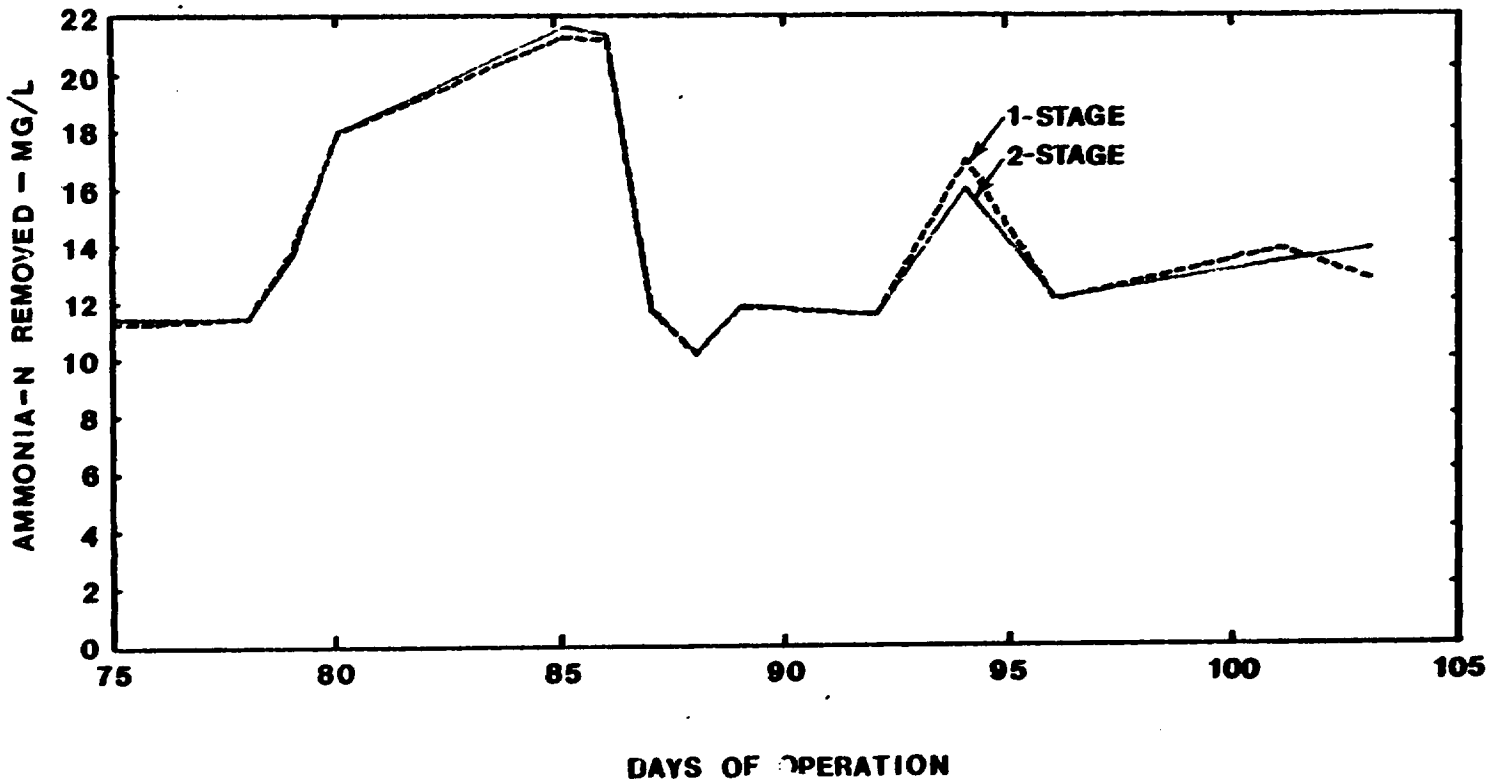


Figure 6.15 Relative Ammonia Removals for 2-Stage and 1-Stage Alkaline Chemical Feed RBC Systems

then the extenders were removed physically and the test was repeated on the same system. Biofilm was present on the discs for both tests. The test data are presented in Figure 6.16 and Table 6.17. The extenders should have increased mixing and trough turbulence. This increase may have accounted for the slightly lower peak tracer concentrations observed in both stages during the tracer study with extenders. Based upon the results of the tracer study, only a slight difference was shown in the hydraulic characteristics of the pilot RBC with and without the extenders.

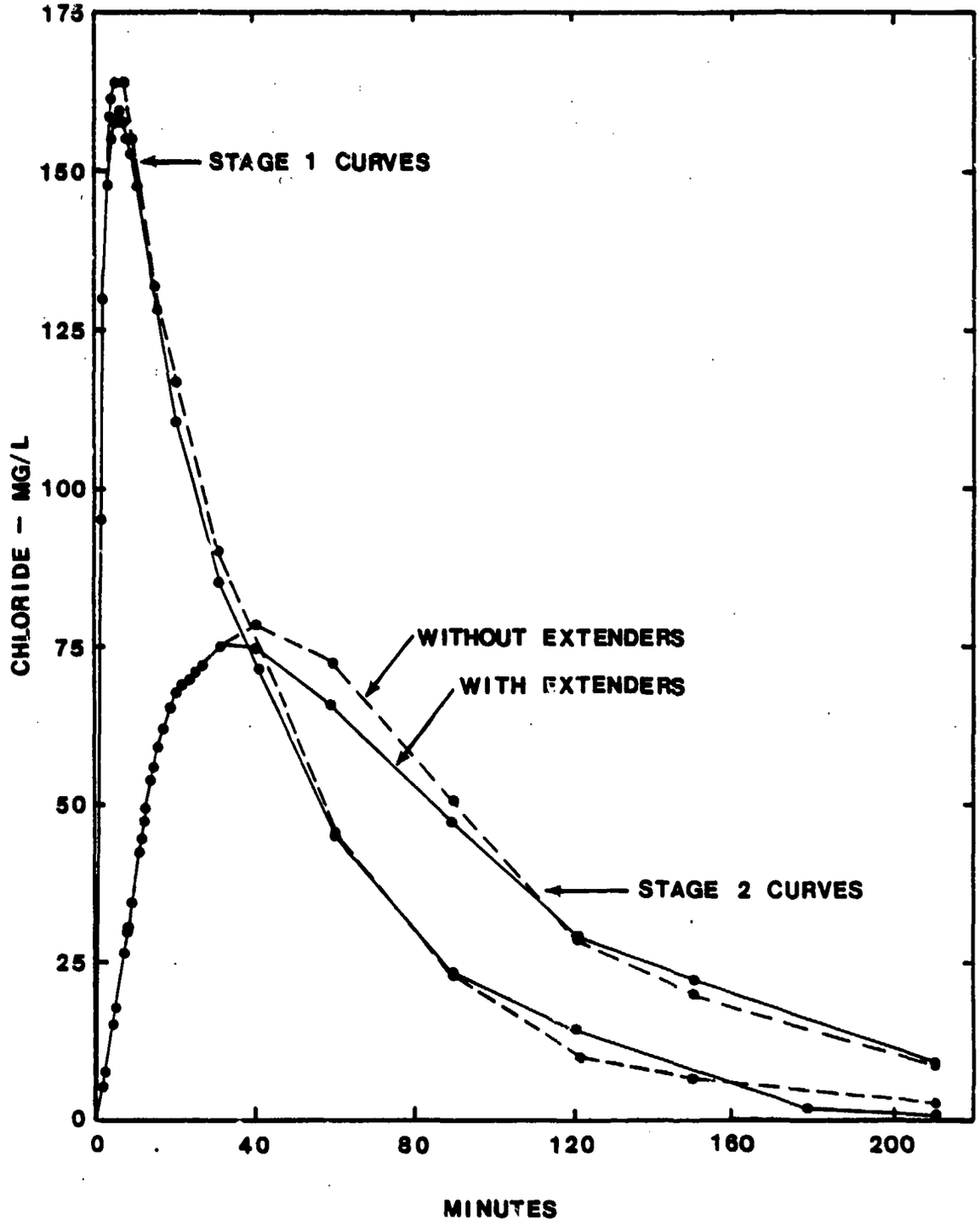


Figure 6.16 Hydraulic Tracer Studies of a 2-Stage RBC System With and Without Extenders

Table 6.17 Hydraulic Characteristics of a 2-Stage RBC System With and Without Extenders

Parameter	With Extenders		Without Extenders	
	Stage 1	Stage 2	Stage 1	Stage 2
Flow Rate, lpm	1.25	1.25	1.22	1.22
RBC Vol., l	35	35	35	35
T = V/Q, min.	28	56	29	57
Tp[a], min.	6.0	35	5.0	40
T_{10}[b], min.	6	22	6	22
T_{50}[c], min.	33	67	32	65
T_{90}[d], min.	100	146	97	143
Disc Biofilm, mg/cm^2	4.30	1.22	4.30	1.22
Tracer Recovery[e], %	100	103	102	106

[a] Tp = time for peak tracer.

[b] T_{10} = time for 10 percent of total tracer.

[c] T_{50} = time for 50 percent of total tracer.

[c] T_{90} = time for 90 percent of total tracer.

[e] Tracer was sodium chloride.

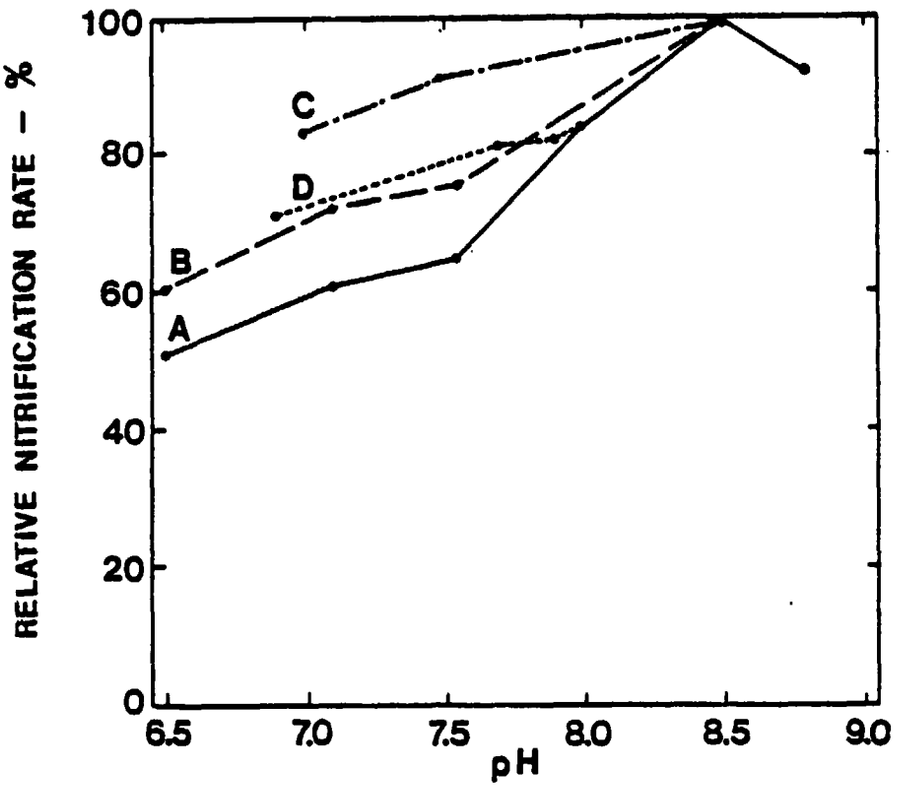
SECTION VII

DISCUSSION AND CONCLUSIONS

7.1 Nitrification and pH

This research examined the short and long term effect of pH upon the nitrification of wastewater within RBC fixed film systems. It evaluated the subject for longer equilibrium periods than any previous research effort which has been reported. The works of other major researchers on the subject are brought into perspective and major points of controversy are resolved.

This research demonstrated that, in the long term, the rate of nitrification within a RBC fixed film system was dependent upon pH. The rate of nitrification increased with increasing pH up to a maximum at pH 8.5 (Figures 4.3, 5.3, and 6.3). This finding is in agreement with many previous research efforts (Table 1.2). The ability of the lower pH systems to develop a nitrifying capacity comparable to the capacity of the higher pH systems, as reported by Haug and McCarty (39) and Huang and Hopson (47), also was observed. However, these high levels of nitrification were not true long term performance levels and did not persist. The lower pH RBC systems lost their ability to maintain a nitrification capacity commensurate with the capacity under optimum pH conditions within approximately five weeks (Figures 5.3 and 6.3). The relative long term nitrification data for the low pH, high pH, and alkaline chemical addition studies are presented in Figure 7.1. These data show that in all cases, up to pH 8.5, the higher pH RBC systems demonstrated higher long term rates of nitrification. The differing levels of performance observed for the different research phases



Legend: Curve A relates the low pH and high pH studies' nitrification performance data obtained during the respective equilibrium periods.

Curve B is the same as A except that the high pH study equilibrium data for the control and pH 8.5 RBCs through Day 130 are the only high pH study data utilized.

Curve C is the first stage equilibrium nitrification performance data for the alkaline chemical addition study for all five RBC systems. Note that between pH 8.4 and pH 8.5 there are three data points between 98 and 100 percent.

Curve D is the second stage equilibrium nitrification performance data for the alkaline chemical addition study for all five RBC systems. Note that at pH 7.9 there are two data points at 82 percent. This second stage data has been related to the pH 8.0 data obtained during the high pH study data.

Figure 7.1 Relative Long Term Nitrification Data Summary for the Low pH, High pH, and Alkaline Chemical Addition Studies

are attributed to variations in biofilm characteristics associated with the slow responsiveness of the microbial systems and low level differences in CBOD concentrations.

The short term response of a nitrifying RBC system to changes in pH was relatively constant from pH 7.0 to pH 8.5 (Figure 5.9). This observation is similar to the findings of Brochardt (16). Below pH 7.0, the adverse effect of pH becomes more pronounced. However, the absolute level of nitrification was dictated by the biofilms' previous history of nitrification performance. RBC systems continued to nitrify at a relatively high rate after the pH had been reduced suddenly. The drop in the level of nitrification, or reversion, took from one to three weeks to occur and was associated with a degradation in the character of the biofilm (Figure 5.10). This result is similar to the findings of Miller (71). These observations indicated that the nitrification performance of the RBC fixed film system was related closely to the characteristics of the biofilm rather than related directly to pH per se.

Except for the first stage of the RBC receiving calcium hydroxide for pH adjustment, the range of alkalinity destruction for all RBC systems in the alkaline chemical addition study was from 6.2 to 7.9 mg $CaCO_3$/l (Table 6.16). This finding is in agreement with alkalinity destruction values commonly found in the literature (97). The level of destruction in the first stage of the calcium hydroxide RBC system was only 3.8 mg $CaCO_3$/l. This result was attributed to a neutralization capacity which developed within the RBC biofilm due to the entrainment of $CaCO_3$ (Table 6.15).

There was no significant difference in the performance of the 2-stage nitrifying RBC systems which received calcium hydroxide, sodium carbonate and sodium hydroxide. The performance levels of the sodium bicarbonate and the control RBC systems were 6 and 16 percent less, respectively (Table 6.5) than those of the other three systems. The use of alkaline chemicals to maintain pH 8.5 in the first stage of a 2-stage nitrifying RBC resulted in the removal of approximately 19 percent more ammonia than the control RBC system.

The amount of unionized ammonia in the wastewater is related to temperature and pH. At 20°C and pH 7.0 there is essentially no unionized ammonia. However, at 20°C and pH 8.5, the amount of ammonia gas increases to approximately 13 percent (97). The ammonia gas is susceptible to stripping from the RBC system. The nitrogen balances for the various research phases (Tables 4.5, 5.4, and 6.7) reveal that the relative amount of nitrogen recovered for each RBC system generally was slightly less for the higher pH systems. This result was attributed to low level ammonia stripping as well as the loss of nitrate due to denitrification within the biofilm.

7.2 Nitrification and Biofilm

Higher levels of nitrification for the RBC systems were associated with greater disc biofilm uniformity. In all cases, except for the pH 8.8 RBC of the high pH study, the higher pH RBC systems maintained greater concentrations of volatile biofilm per unit of RBC disc area (Sections 4.3.2, 5.3.2, 6.3.2, and Appendix B).

The loss of biofilm from the RBC disc surface did not follow the traditionally accepted sloughing pattern. Biofilm did not slough from

the disc surface outward. The dominant pattern of biofilm loss was from the biofilm surface inward. This loss was due to hydraulic shear at the biofilm surface. The RBC disc biofilm characteristics changed with time. The initial biofilm was uniform in texture and tan to bronze in color. The biofilm went through an aging process wherein the biofilm became darker and the texture became less uniform; the lower the pH, the less uniform the biofilm. The disc biofilm was affected greatly by low level changes in CBOD. The maximum rates of nitrification for individual RBC stages were not associated with the maximum biofilm concentrations on the discs. Disc biofilm continued to develop after the individual RBC stages achieved their maximum rate of nitrification. Based upon disc biofilm concentrations, which were present at the time maximum nitrification was observed initially and an estimate for microorganism density of 95 mg/cm^3 (58), the active biofilm thickness were estimated to be from 50 to 300 microns.

The amounts of biofilm which developed on the walls of both stages of the control were less than the amounts of biofilm developed on the corresponding rotating discs. This observation indicated that the diffusional characteristics associated with the products and reactants of the nitrification process played a greater role in biofilm development than did hydraulic shear alone (Figures 6.7 and 6.8).

The numbers of nitrifying bacteria within these nitrifying RBC systems were relatively high compared to previously reported populations for other nitrifying environments (Table 1.3). The bacterial enumerations revealed that the concentration of heterotrophic bacteria and ammonia-oxidizing bacteria were generally of the same order of magnitude. The concentrations of nitrite-oxidizing bacteria were

approximately of an order of magnitude less (Figures 4.8, 5.8, and 6.9). The elevated pH RBC biofilms, which had enhanced nitrification capacities, had higher nitrifying bacterial populations than the lower pH RBC biofilms. The ammonia-oxidizing bacteria generally were favored over the nitrite-oxidizing bacteria with respect to increasing pH. (Figure 4.8, 5.2, 5.8, 6.9). Greater heterotrophic growth and more rapid biofilm development was observed to occur at elevated pH levels (Figures 5.6, 6.11, and 6.12).

The level of nitrifying bacteria activity demonstrated that sufficient populations of ammonia-oxidizing bacteria were present within the RBC systems to oxidize much more ammonia than observed (Table 6.13). The distribution of viable nitrifying bacteria and heterotrophic bacteria within the control RBC stages (Table 6.14) revealed that most of the heterotrophic and nitrifying bacteria were on the RBC discs. Less than 5 percent were on the walls of the RBC or within the wastewater. However, the distribution of bacteria cannot be interpreted as an accurate representation of overall microbial activity.

Snails which existed within the high rate trickling filter readily inhabited the walls of those RBCs which were maintained between pH 7.0 and 8.0. They could be purged from the system by elevating the pH of the RBC stage to pH 8.5. These snails did not affect treatment efficiency but did constitute a nuisance. Other secondary predators within the biofilm may have had an important impact on the RBC treatment efficiency. Torpey (93) observed bare spots in the nitrifying stages of an RBC facility treating domestic wastewater at slightly above neutral pH conditions. This loss of biofilm was attributed to predator

populations within the RBC biofilm. The high pH environments of this research may not have favored such secondary predator populations. This condition may account for the greater biofilm uniformity at the higher pH levels. The length of time for such secondary predator populations to become established may explain why the neutral pH RBC environments initially perform as well as the optimum pH environments and why five weeks were needed to achieve equilibrium.

SECTION VIII

DESIGN RECOMMENDATIONS

The following RBC design recommendations are made regarding alkaline chemical feed systems for the enhancement of nitrification of domestic wastewater within RBC systems.

1. The optimum pH for RBCs with alkaline chemical feed systems is pH 8.5. The alkaline chemical should be added directly into the RBC troughs. The addition of alkaline chemical to the RBC stages should be at such intervals along the RBC trough to ensure that pH variations within the trough do not exceed ± 0.2 pH units. This design will ensure uniformity of treatment and preclude inorganic solids buildup due to localized pH excursions. Chemical addition points should be located to ensure that the effluent pH level accurately represents the RBC stage pH level. The alkaline chemical addition should be controlled with a pH controller feedback system which measures in-stage pH. A redundant pH controller backup system is necessary to protect against primary controller failure.

2. The selection of the appropriate alkaline chemical for pH control should be based solely upon feeding and economic considerations and not inorganic carbon content. In general, soda ash and lime will be the alkaline chemicals of choice. Where the nitrifying RBC system does not include a clarification step, soda ash should be used. Lime addition produces sufficient inorganic suspended solids to require final clarification following nitrification.

3. The alkaline chemical dosage should be determined from titration curves developed for the specific wastewater over the alkalinity

extremes expected. Short term and long term variations in hydraulic and organic loadings will result in the initial nitrifying stage shifting between two or more RBC stages. Therefore, the alkaline chemical should be added to at least two of the initial RBC stages where the nitrification process starts. Preferably, all of the RBC stages where nitrification may occur should have alkaline chemical feed capability.

4. Rates of disc rotation and/or aeration should be adjusted to maintain DO levels above 1 mg/l. This procedure will protect against DO limitations and chemical wastage.

5. Compliance and/or contractual performance testing should not be attempted for a minimum of six weeks following startup in order to allow the nitrifying system to reach dynamic equilibrium.

SECTION IX

FUTURE RESEARCH

This research has indicated related areas which require additional investigation. Future research efforts should be designed to:

1. Evaluate full scale nitrifying RBC facilities utilizing alkaline chemical addition for pH control on a long term basis. Such facilities should be operated for a minimum of six months under equilibrium conditions for each alkaline chemical employed.

2. Characterize the RBC biofilm with respect to the long term pH effect on secondary microbial populations and their relationship to RBC nitrification performance.

3. Evaluate the impact of low level fluctuations in CBOD and ammonia-nitrogen upon the character of the biofilm and its relationship to the RBC nitrification process.

4. Evaluate the ability of elevated pH environments to enhance heterotrophic activity and improve CBOD removal.

5. Develop methodologies to exploit the unused microbial nitrifying activity potential within the biofilm.

6. Examine the long term effect of calcium carbonate buildup on the RBC discs where lime feed is utilized.

7. Evaluate intermittent pH control and intermittent alkaline chemical addition in order to determine the low pH tolerance of such systems before nitrification performance deteriorates.

8. Evaluate low level injections of wastewater into nitrifying RBC stages to raise the CBOD level and possibly enhance the nitrifying biofilm characteristics.

BIBLIOGRAPHY

1. Adams, C. E. and W. W. Eckenfelder, "Nitrification Design Approach for High Strength Ammonia Wastewater," Jour. Water Poll. Control Fed., 49, 413 (1977).

2. Alexander, Martin, and Francis E. Clark, "Nitrifying Bacteria," Methods of Soil Analysis - Part 2, C. A. Clark (ed.), Am. Soc. Agron., Madison WI (1965).

3. Anderson, J. H., "Oxidation of Ammonia by Nitrosomonas," Biochemical Jour., 95, 688-698 (1965).

4. Anderson, J. H., "The Metabolism of Hydroxylamine to Nitrite by Nitrosomonas," Biochemical Jour., 91, 8-17 (1964).

5. Anthonisen, A. C. et al., "Inhibition of Nitrification by Ammonia and Nitrous Acid," Jour. Water Poll. Control Fed., 48, 835 (1976).

6. Antoine, R. L. et al., "Evaluation of a Rotating Disc Wastewater Treatment Plant," Jour. Water Poll. Control Fed., 46, 498 (1974).

7. Antoine, R. L., Fixed Biological Surfaces - Wastewater Treatment, CRC Press, West Palm Beach, Fla. (1976).

8. Antoine, R. L., "Nitrification of Activated Sludge Effluent: Bio-Surf Process Part 1," Water and Sewage, 11, 44 (1974).

9. Antoine, R. L., "Nitrification of Activated Sludge Effluent: Bio-Surf Process - Part 2," Water and Sewage, 12, 54 (1974).

10. Antoine, R. L., "Nitrogen Control with the Rotating Bio Contactor," Autotrol Corporation, (undated).

11. Autotrol Corp., Wastewater Treatment System Design Manual, Milwaukee, WI (1979).

12. Belser, L. W., "Nitrate Reduction to Nitrite, A Possible Source of Nitrite for Growth of Nitrite-Oxidizing Bacteria," Appl. and Env. Microb., 34, 403-410 (1977).

13. Belser, Lawrence W., "Population Ecology of Nitrifying Bacteria," Ann. Rev. Microb., 33, 309-333 (1979).

14. Benninger, Robert N. and Joseph H. Sherrad, "Nitrification and Alkalinity Relationships in Activated Sludge," Jour. Water Poll. Control Fed., 50, 2132 (1978).

15. Boon, B. and H. Laudelot, "Kinetics of Nitrite Oxidation by Nitrobacter Winogradski," J. Biochem., 85, 440-447 (1962).

16. Borchardt, J. A., et al., Nitrification of Secondary Municipal Waste Effluents by Rotating Bio-Discs, USEPA Grant No. R803407, Cincinnati, Ohio, (1978).

17. Bridle, T. R., et al., "Operation of Full-Scale Nitrification-Denitrification Industrial Waste Treatment Plant," Jour. Water Poll. Control Fed., 51, 127 (1979).

18. Buswell, A. M., et al., "Laboratory Studies on the Kinetics of the Growth of Nitrosomonas with Relation to the Nitrification Phase of the BOD Test, Appl. Microb., 2, 21-25 (1954).

19. Characklis, W. G. and M. G. Trulear, "Dynamics of Microbial Film Processes," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 365-408 (1980).

20. Chesner, W. H. and J. J. Iannone, "Current Status of Municipal Wastewater Treatment with RBC Technology in the United States," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 53-70 (1980).

21. Costerton, J. W., et al., "How Bacteria Stick," Sci. Amer., 238, 86-95 (1978).

22. Crawford, Paul M., "Use of Rotating Biological Contactors for Nitrification at the City of Guelph Water Pollution Control Plant, Guelph, Ontario, Canada," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 1247-1274 (1980).

23. Davis, Judith A., The Microbiology of an Activated Sludge Wastewater Treatment Plant, Masters Thesis, Pennsylvania State University (1972).

24. Delwiche, C. C. and M. S. Finstein, "Carbon and Energy Sources for the Nitrifying Autotrophic Nitrobacter," Jour. Bact., 90, 102-107 (1965).

25. DiSalvo, Richard M. and Joseph H. Sherrad, "The Stimulation of Nitrification at Low Nickel Concentrations," Paper Presented at 12th Mid-Atlantic Industrial Waste Conference (1980).

26. Doherty, Michael C., Heterotrophic Populations in a Rotating Biological Contactor Nitrification System, Masters Paper, Department of Civil Engineering, Pennsylvania State University (1981).

27. Dolloff, F. Bishop, et al., Single Stage Nitrification - Denitrification, EPA 670/2-75-051 (1975).

28. Downing, A. L. and G. Knowles, "Population Dynamics in Biological Treatment Plants," Advances in Water Pollution Research: Proceedings of 3rd International Conference in Munich, Germany, Vol 2, (1966).

29. Downing, A. L., et al., "Effect of Inhibitors on Nitrification in the Activated Sludge Process," Jour. of Inst. Sew. Purif., 537 (1964).

30. Downing, A. L., et al., "Nitrification in the Activated Sludge Process," Jour. of Inst. of Sew. Purif., 130 (1964).

31. Engel, M. S. and M. Alexander, "Growth and Autotrophic Metabolism of Nitrosomonas Europaea," Jour. Bact., 76, 217-222 (1958).

32. Famularo, Jack, et al., "Application of Mass Transfer to Rotating Biological Contactors," Jour. Water Poll. Control Fed., 50, 653 (1978).

33. Finstein, M. S. and V. A. Matulewich, Nitrification Potential of River Environments, A-039-NJ, Dept. of Sci., Cook College, Rutgers University (1977).

34. Finstein, M. S., et al. Evaluation of Nitrification in the Water Column of the Passaic River, A-053-NJ, Dept. of Sci., Cook College, Rutgers University (1977).

35. Friedman, A. A., et al., "Effect of Disc Rotational Speed on Biological Contactor Efficiency," Jour. Water Poll. Control Fed., 51, 2678 (1979).

36. Gasser, James A., et al., "Fixed Film Nitrification of Secondary Effluent," Paper Presented at Envir. Engr. Research Conf., Pennsylvania State University (1974).

37. Gaudy, Anthony F. and Elizabeth T. Gaudy, Microbiology for Environmental Scientists and Engineers, McGraw-Hill, New York (1980).

38. Ghiorse, William C. and Martin Alexander, "Nitrifying Populations and the Destruction of Nitrogen Dioxide in Soil," Microb. Ecol., 4, 233-240 (1978).

39. Haug, Robert T. and Perry L. McCarty, "Nitrification with Submerged Filters," Jour. Water Poll. Control Fed., 44, 2086 (1972).

40. Heidman, James A., et al., Carbon, Nitrogen, and Phosphorus Removal in Staged Nitrification - Denitrification Treatment, USEPA 670/2-75-052, (1975).

41. Hewitt, T., Nitrification of a Secondary Municipal Effluent Using a Rotating Biological Contactor, Research Publication No. 71, Ontario Ministry of the Environment (1978).

42. Hitdlebaugh, J. A. and R. D. Miller, "Full-Scale Rotating Biological Contactor for Secondary Treatment and Nitrification," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, (1980).

43. Hitdlebaugh, John A., Phase I, Water Quality Engineering Special Study No. 32-24-0116-79, Sewage Treatment Plant Evaluation, Summer Conditions, Fort Knox, Kentucky, 14-24 August and 25-29 September, 1978, US Army Envir. Hygiene Agency (1979).

44. Hoag, George E., et al., "Microfauna and RBC Performance: Laboratory and Full Scale," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 167-188 (1980).

45. Hoffman, T. and H. Lee, "The Biochemistry of the Nitrifying Organisms, Part 4. The Respiration and Intermediary Metabolism of Nitrosomonas," Jour. of Biochem., 54, 579-583 (1953).

46. Huang, Ching-San and Ninnian E. Hopson, "Nitrification Rate in Biological Processes," Jour. ASCE EED, Apr, 409 (1974).

47. Huang, C. S. and N. E. Hopson, "Temperature and pH Effect on the Biological Nitrification Process," Paper Presented at N.Y. Water Poll. Control Ass'n Winter Meeting, N.Y. City (1974).

48. Hutton, W. C. and S. A. LaRocca, "Biological Treatment of Concentrated Ammonia Wastewaters," Jour. Water Poll. Control Fed., 47, 989 (1975).

49. Hynek, Robert J. and Hiroshi Iemura, "Nitrogen and Phosphorus Removal with Rotating Biological Contactors," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 295-324 (1980).

50. Ito, K. and T. Matsuo, "The Effect of Organic Loading on Nitrification in RBC Wastewater Treatment Processes," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 1165-1176, (1980).

51. Jenkins, Charles R., et al., "Nitrification in a Pure Oxygen Fixed Film Reactor," 12th Mid Atlantic Industrial Waste Conference, Bucknell University (1980).

52. Jirka, Andrea M. and Mark J. Carter, "Micro Semi-Automated Analysis of Surface and Wastewaters for Chemical Oxygen Demand," Analytical Chemistry, 47, 1379-1402 (1975).

53. Jirka, Andrea M., et al., "Ultramicro Semi-Automated Method for the Simultaneous Determination of Total Phosphorus and Total Kjeldahl Nitrogen in Wastewaters," USEPA Region V, Chicago (undated).

54. Joost, Bob and Mike Vesio, "Evaluation of a Rotating Biological Disc in a Sewage Treatment Process in a Package Plant Application," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 349-364 (1980).

55. Kaltreider, Randall L., The Comparative Microbiology of a Phostrip and Conventional Activated Sludge Pilot Plant Wastewater Treatment System, Masters Thesis, Pennsylvania State University (1979).

56. Kholdebarin, Bahman and J. J. Dertli, "Effects of pH and Ammonia on the Rate of Nitrification of Surface Waters," Jour. Water Poll. Control Fed., 49, 1688 (1977).

57. Kim, Byung Joon and Alan H. Molof, "Physical Factors in RBC Oxygen Transfer," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 87-102 (1980).

58. Kincannon, Don F. and Steve Groves, "Role of Suspended Solids in the Kinetics of RBC Systems," Proceedings: First National Symposium/Workshop on Rotating Biological Contactors, Champion, PA (1980).

59. Kinner, Nancy E. and Paul L. Bishop, "High Salinity Wastewater Treatment Using Rotating Biological Contactors," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 259-268 (1980).

60. LaBeda, David P. and Martin Alexander, "Effects of SO_2 and NO_2 on Nitrification in Soil," Jour. Environ. Qual., 7, 523-526 (1978).

61. LaMotta, E. J. and R. F. Hickey, "Factors Affecting Attachment and Development of Biological Films on Solid Media," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 803-828 (1980).

62. Loveless, J. E. and H. A. Painter, "The Influence of Metal Ion Concentrations and pH Value on the Growth of a Nitrosomonas Strain Isolated from Activated Sludge, Jour. Gen. Microb., 52, 1-14 (1968).

63. Lue-Ling, Cecil, et al., "Biological Nitrification of Sludge Supernatant by Rotating Discs," Jour. Water Poll. Control Fed., 48, 25-39 (1976).

64. Matulewich, V. A. and M. S. Finstein, "Distribution of Autotrophic Nitrifying Bacteria in a Polluted River (the Passaic)," Appl. and Environ. Microbiol., 35, 67-71 (1977).

65. Matulewich, V. A., et al., "Length of Incubation for Enumerating Nitrifying Bacteria Present in Various Environments," Appl. Microb., 29, 265-268, (1975).

66. McCarty, P. L., "Anaerobic Waste Treatment Fundamentals," Public Works, 91-94, (Oct, 1964).

67. McGhee, Mary Frances, Fundamental Studies of the Nitrification Process, Ph.D. Thesis, Department of Civil Engineering, University of Kansas, (1975).

68. McHarness, Donald D. and Perry L. McCarty, Field Study of Nitrification with the Submerged Filter, USEPA No. R2-73-158 (1978).

69. Metcalf and Eddy Inc., Wastewater Engineering: Treatment, Disposal, Reuse, McGraw-Hill, New York (1979).

70. Meyerhof, O., "Untersuchungen uber den Atmungsvorgany Nitrifizierenden Bakterien," Pflugers Archges Physiol., 166, 240-280 (1917).

71. Miller, Roy D., et al., Rotating Biological Contactor Process for Secondary Treatment and Nitrification Following a Trickling Filter, Technical Report 7805, US Army Medical Bioengineering Research and Development Laboratory (1979).

72. Mueller, James A., et al., "Nitrification in Rotating Biological Contactors," Jour. Water Poll. Control, 52, 688-710 (1980).

73. Mulbarger, M. C., The Three Sludge System for Nitrogen and Phosphate Removal, Advanced Waste Treatment Research Laboratory, Office of Research and Monitoring, Environmental Protection Agency (1972).

74. Murphy, Keith L., et al., "Nitrogen Control: Design Considerations for Supported Growth Systems," Jour. Water Poll. Control Fed., 549-557 (Apr, 1977).

75. Olem, Harvey, Rotating-Disc Biological Oxidation of Ferrous Iron in Acid Mine Drainage Treatment, Ph.D. Thesis, Department of Civil Engineering, Pennsylvania State University (1978).

76. O'Shaughnessy, James C. and Frederic C. Blanc, Biological Nitrification and Denitrification Using Rotating Biological Contactors, Publication No. 97, Water Resources Research Center, University of Massachusetts, Amherst (1978).

77. Ouyang, C. F., "The Characteristics of Rotating Biological Contactor (RBC) Sludge," Proceedings: First National Symposium/Workshop on Rotating Biological Contactor Technology, Champion, PA, 189-204 (1980).

78. Painter, H. A., "A Review of Literature on Inorganic Nitrogen Metabolism," Water Research, 4, 393-450 (1970).

79. Painter, H. A., "Microbial Transformations of Inorganic Nitrogen," Proceedings: Conference on Nitrogen as a Wastewater Pollutant, Copenhagen, Denmark (Aug. 1975).

80. Perkin-Elmer Co., Analytical Methods for Atomic Adsorption Spectrophotometry (1976).

81. Rowe, R., et al., "Microtechnique for Most-Probable-Number Analysis," Appl. and Environ. Microb., 33, 625-680 (1978).

82. Saunders, F. Michael and Rodney L. Pope, Nitrification with Rotating Biological Contactor Systems, ERC 06-78, School of Civil Engineering and Environmental Research Center, Georgia Tech. (1978).

83. Searce, Steven, et al., "Prediction of Alkalinity Changes in the Activated Sludge Process," Jour. Water Poll. Control Fed., 52, 399-405 (1980).

84. Sherrard, Joseph H., "Destruction of Alkalinity in Aerobic Biological Wastewater Treatment," Jour. Water Poll. Control Fed., 48, 1835 (1976).

85. Smith, E. D. and J. T. Bandy, "A History of the Rotating Biological Contactor Process," Proceedings: First National Symposium/ Workshop on Rotating Biological Contactor Technology, Champion, PA, 11-26 (1980).

86. Srinath, Embar G., et al. "Nitrifying Organism Concentration and Activity," ASCE Jour. EED, 449 (Apr 1976).

87. Srna, Richard F. and Anne Bagley, "Kinetic Response of Perturbed Marine Nitrification Systems," Jour. Water Poll. Control Fed. 47, 473 (1975).

88. Standard Methods for the Examination of Waste and Wastewater, 14th Edition, Amer. Public Health Ass'n, New York (1976).

89. Stover, E. L. and D. F. Kincannon, "One-Step Nitrification and Carbon Removal," Water and Sewage Works, 122 (1975).

90. Strom, P. F., et al., "Concentrations of Nitrifying Bacteria in Sewages, Effluents, and A Receiving Stream and Resistance of these Organisms to Chlorination," Appl. and Environ. Microb. 31, 731-737 (1976).

91. Stratta, J. M. and B. J. Davis, Wastewater Treatment Plant Effluent Characterization and Treatment Plant Study No. 66-0157-77, Fort Lewis, Washington, 25 July - 4 August, 1977, US Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD (1977).

92. Taylor, C. B. "The Nutritional Requirements of the Predominate Bacterial Flora of Soil," Proc. Soc. Appl. Bact., 14, 101-111 (1951).

93. Torpey, W. et al., "Rotating Discs with Biological Growths Prepare Wastewater for Disposal or Reuse," Jour. Water Poll. Control Fed., 43, 2181 (1971).

94. Turner, Nash Beth, "Biological Nitrification - One Industries Approach," 11th Mid-Atlantic Industrial Waste Conference, Pennsylvania State University, (July 1979).

95. Unz, Richard F. and Judith A. Davis, "Microbiology of Combined Chemical-Biological Treatment," Jour. Water Poll. Control Fed. 47, 185 (1975).

96. USEPA, Methods for Chemical Analysis of Water and Wastes, Washington, D.C. (1979).

97. USEPA, Process Design Manual for Nitrogen Control (1975).

98. US General Accounting Office, Costly Wastewater Treatment Plants Fail to Perform as Expected, No. CED-81-9, Washington, D.C. (1980).

99. Verstrate, W., et al., "Adaption to Nitrification of Activated Sludge Systems Treating Highly Nitrogenous Waters," Jour. Water Poll. Control Fed., 49, 1604 (1977).

100. Villemonte, James A. and Gerald A Rohlich, "Hydraulic Characteristics of a Circular Sedimentation Basin," 17th Purdue Industrial Waste Conference, 682-702 (1962).

101. Wanatabe, Y., et al., "Nitrification Kinetics in a Rotating Biological Disk Reactor," Prog. Water Tech., 12, 233-251 (1980).

102. Watson, Stanley R., "Part 12 - Gram-Negative Chemolithotrophic Bacteria," Bergy's Manual of Determinative Bacteriology, 8th Edition, 450-456 (1974).

103. Weng, Cheng-Nan and Alan H. Molog, "Nitrification in a Biological Fixed Film Rotating Disc System," Jour. Water Poll. Control Fed. 46, 1674 (1974).

104. Wild, Harry E., et al., "Factors Affecting Nitrification Kinetics," Jour. Water Poll. Control Fed. 43, 1845 (1971).

105. Young, James C., et al., "Packed-Bed Reactors for Secondary Effluent BOD and Ammonia Removal," Jour. Water Poll. Control Fed., 47, 46 (1975).

APPENDIX A

ANALYTICAL PROCEDURES

Wastewater Analysis

Wastewater sampling. Timed grab samples taken during the evening hours, which were representative of maximum wastewater pollutant concentrations, were composited with timed grab samples taken during the early morning hours, which were representative of minimum wastewater pollutant concentrations. The characteristics of samples taken in this manner correlated well with time (and flow) composited samples taken independently with compositing samplers (N-Con, New Rochelle, N.Y.).

Alkalinity. Alkalinity measurements were performed utilizing an Orion 901 Ionanalyzer in conjunction with a combination glass electrode in accordance with the procedure described in Standard Methods (88) for waters containing phosphorus, i.e., endpoint pH 4.5.

Ammonia Nitrogen. NH_3-N was determined colorimetrically with a Technicon Autoanalyzer II utilizing the phenate method in Standard Methods (88).

Total Kjeldahl Nitrogen. TKN was determined colorimetrically with a Technicon Autoanalyzer II utilizing the phenate method described by Jirka (53).

Nitrite Nitrogen. NO_2-N was determined colorimetrically with a Technicon II utilizing the cadmium reduction method described in Standard Methods (88). The copper-cadmium reduction column was removed to eliminate nitrate from the analysis.

Nitrite plus Nitrate Nitrogen. (NO_2+NO_3)-N was determined colorimetrically with a Technicon Autoanalyzer II utilizing the cadmium reduction method described in Standard Methods (88).

Total Phosphorus. Total phosphorus was determined in accordance with the single reagent digestion method described by Jirka (53) and analyzed on a Technicon Autoanalyzer II.

Orthophosphate Phosphorus. Orthophosphate was determined colorimetrically with a Technicon Autoanalyzer II utilizing the single reagent method described in Methods of Chemical Analysis of Water and Wastes (96).

Chlorides. An Orion Model 96-17 probe with an Orion Ionanalyzer Model 801 was used to determine chloride concentrations.

Sulfates. Sulfates were determined with a Hach Model 2100A Turbidimeter in accordance with the turbidimetric method described in Standard Methods (88).

Dissolved Oxygen. Wastewater DO was measured on grab samples with a YSI Model 54A Oxygen Meter at the RBC research facility.

Temperature. Measurements of wastewater temperature were made with the YSI Model 54A Oxygen Meter and Fischer Scientific thermometers. All thermometers were precalibrated to the same standard.

pH. Determinations of wastewater pH were made at the RBC research facility with a Fischer Accumet pH Controller Model 650 and a Beckman Zeromatic pH Meter.

Total and Volatile Suspended Solids. Wastewater samples were filtered through Whatman (Reeve Angel) glass fiber filters (5.5 cm) and analyzed for total and volatile suspended solids according to Standard Methods (88).

Biochemical Oxygen Demand. Total 5 day BOD was determined according to Standard Methods (88). Nitrification was inhibited with N-Serv manufactured by Hach Co. and DO was determined by means of the Winkler procedure according to Standard Methods (88). Soluble BOD analyses were performed upon samples filtered through glass fiber filters. All data presented in the text are 5 day soluble inhibited BOD values unless stated otherwise.

Metals. All metal analyses were performed according to USEPA methodology (96) on a Perkin Elmer Atomic Adsorption Model 703 with Graphite Furnace Model 2200 (80).

Microbial Analysis

RBC Biofilm Sampling. The biofilm sampling procedures were a modification of those described by Olem (75). RBC disc biofilm was sampled utilizing premeasured mylar wedges (49.5 cm^2) glued in place and radiated from the center to the edge of flat plexiglass discs attached to the RBC shaft. Wall biofilm was sampled utilizing premeasured mylar squares (25 cm^2) which were glued to the RBC trough walls. The biofilm samples were scrapped from the wedges and mixed with 100 ml of sterile culture media phosphate buffered water (88). The samples were blended in the cold for one minute at approximately 19,000 rpm with a single speed Waring blender (64, 75, 90). The homogeneous mixture then was apportioned for microbiological, physical, and chemical analysis.

Heterotrophic Bacteria. The enumeration of heterotrophic bacteria was accomplished by spread plating serial dilutions of Modified Taylor's Media (92) and incubating for 10 days at 28°C. The modified Taylor's Media was prepared according to Kaltreider (55) using activated sludge

extract. The media contained per liter: yeast extract (DIFCO), 0.5 g; tryptone (DIFCO), 0.5 g; K_2HPO_4, 0.4 g; NaCl, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; $FeCl_3$, 0.01 g; 1 ml of trace metals solution and activated sludge extract, 250 ml. The activated sludge extract was prepared by autoclaving a volume of activated sludge for 20 minutes at 121°C, centrifuging at 7,000 rpm for 15 minutes in a Sorval Model RC2-B refrigerated centrifuge; and filtering the supernatant through Whatman No. 4 filter paper. The trace metals solution contained $ZnSO_4 \cdot 7H_2O$, 0.0704 g; $CuSO_4 \cdot 5H_2O$, 0.0068 g; $MnSO_4 \cdot H_2O$, 0.0677 g; H_3BO_3, 0.1201 g; $CoCl_2 \cdot 6H_2O$, 0.0565 g; and $Na_2MoO_4 \cdot 2H_2O$, 0.39 g. The modified Taylor's media received 15 g of granulated agar per liter and then was autoclaved for 15 minutes at 121°C prior to pouring into Petri dishes. An innoculum of 0.1 ml of each dilution was spread evenly upon each dish.

Ammonia-Oxidizing Bacteria. The most probable numbers (MPN) of ammonia-oxidizing bacteria were determined using a modification of the Nitrosomonas MPN technique of Alexander and Clark (2) which was reported by LaBeda and Alexander (60) as well as Rowe (81). This technique utilized duplicate 5-tube MPN tests using 96-well, flat-bottomed, Microtest II tissue culture plates (Falcon Plastics, Oxnard, Calif.) containing 0.2 ml per well of Nitrosomonas media described by Alexander and Clark (2). Replicate 0.1 ml portions were transferred to 10 wells for each dilution; a control well also was innoculated before and after the 10 test wells in order to provide a control against contamination for each dilution and to protect against evaporation. The tissue culture plates were sealed with pressure sensitive tape (Falcon Plastics,

Oxnard, Calif.) and incubated for 28 days at 28°C. Griess-Ilosvay reagent (2) which produces a pink to an orange color in the presence of nitrite was used as the indicator. Due to the production of a light pink color when the indicator reagent has time to react with the media within the wells, the end point frequently is difficult to ascertain. Therefore, the MPN values were obtained by two individuals independently evaluating each plate after 10 minutes of color development. Good agreement has been reported between MPN values determined by this microtechnique and the standard tube technique (60, 81).

Nitrite-Oxidizing Bacteria. The MPN values for nitrite-oxidizing bacteria were determined using a modification of the Nitrobacter MPN technique of Alexander and Clark (2) which was reported by LaBeda and Alexander (60) and Ghiorse and Alexander (38). This technique utilized duplicate 5-tube MPN tests using 96-well, flat-bottomed, Microtest II tissue culture plates (Falcon Plastics, Oxnard, Calif.) containing 0.2 ml per well of Nitrobacter media described by Alexander and Clark (2). Replicate 0.1 ml portions of each dilution were transferred to 10 wells. A control well also was innoculated before and after the 10 test wells to provide a control against contamination for each dilution and to protect against evaporation. The tissue culture plates were sealed with pressure sensitive tape (Falcon Plastics, Oxnard, Calif.) and incubated for 28 days at 28°C. Griess-Ilosvay reagent (2) was used as the indicator. A distinct pink color developed in the presence of nitrite. The MPN values were obtained by two individuals independently evaluating each plate after 10 minutes of color development. Good agreement has been reported between MPN values determined by this microtechnique and

the standard tube technique by LaBeda and Alexander (60) while Ghiorse and Alexander (38) reported that microtechnique MPN values were three to four times higher.

RBC Biofilm Solids. Total and volatile biofilm solids concentrations were determined with both glass fiber filters and porcelain evaporating dishes in accordance with Standard Methods (88). Data obtained with the porcelain evaporating dish were corrected to the filter technique. The correction was based upon 24 paired data values which related total and volatile solids values. Total nitrogen and metals were determined according to the total Kjeldahl nitrogen and metals procedures described above.

Biofilm Acid Neutralization Capacity. The biofilm acid neutralization capacity was determined by placing a sample of biofilm into 50 ml of distilled water and titrating with a standardized sulfuric acid solution to a pH 4.5 endpoint. If the pH migrated upward with time, the titration was repeated until no movement from the pH 4.5 endpoint was observed.

APPENDIX B: RBC PHOTOGRAPHS

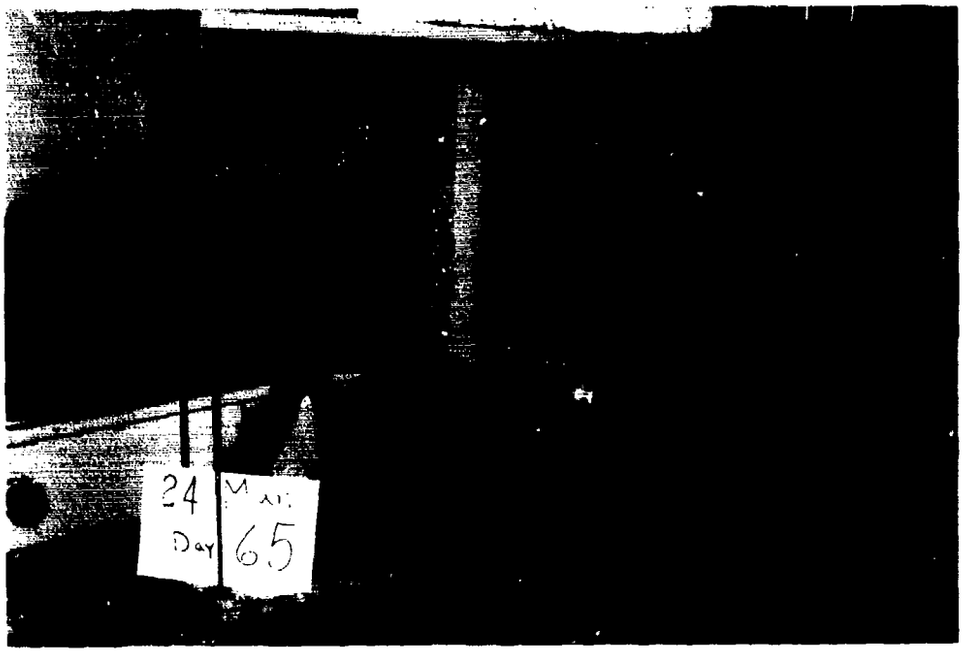


Figure B.1 RBC Biofilms Developed Under Low pH Conditions - (Left to Right) pH 7.5, pH 7.1, pH 6.5, and pH 6.3/6.7

Figure B.2 RBC Biofilms Developed Under High pH Condition - (Left to Right) pH 7.6, pH 8.0, pH 8.5, and pH 8.8

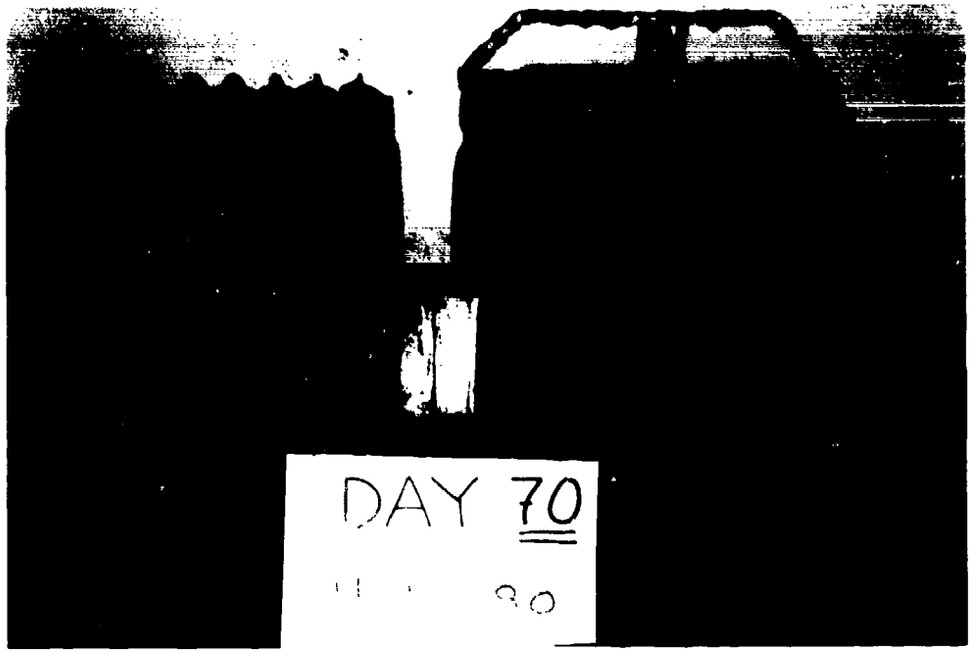


Figure B.3 First Stage (Right) and Second Stage (Left) RBC Biofilms of Control RBC System After 70 Days of Operation During the Alkaline Chemical Addition Study

Figure B.4 First Stage RBC Biofilm for the Calcium Hydroxide RBC System After 70 Days of Operation During the Alkaline Chemical Addition Study

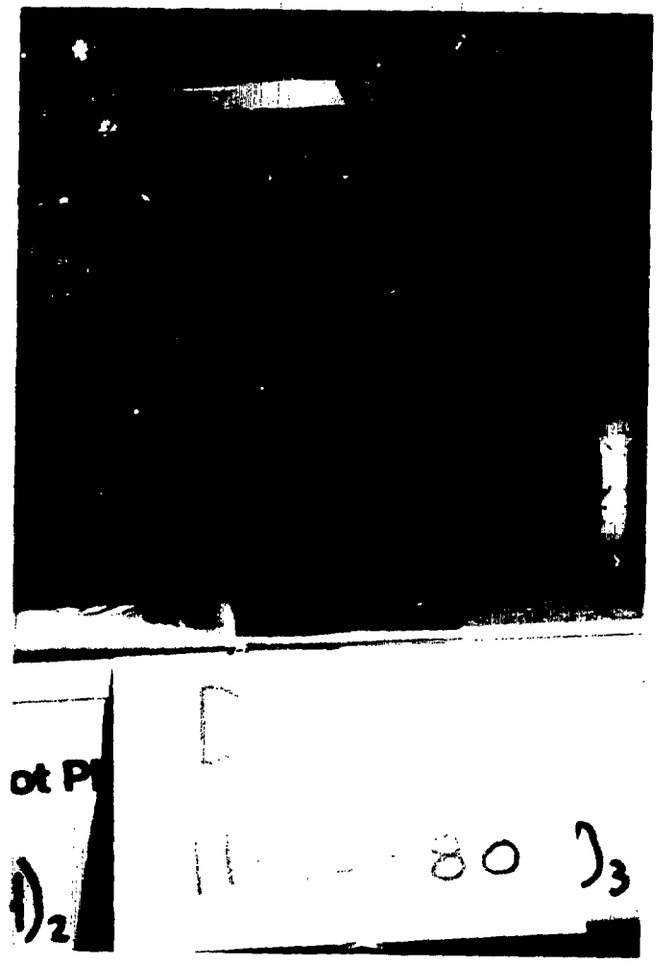


Figure B.5 First Stage RBC Biofilm for the Sodium Carbonate RBC System After 70 Days of Operation During the Alka_ine Chemical Addition Study

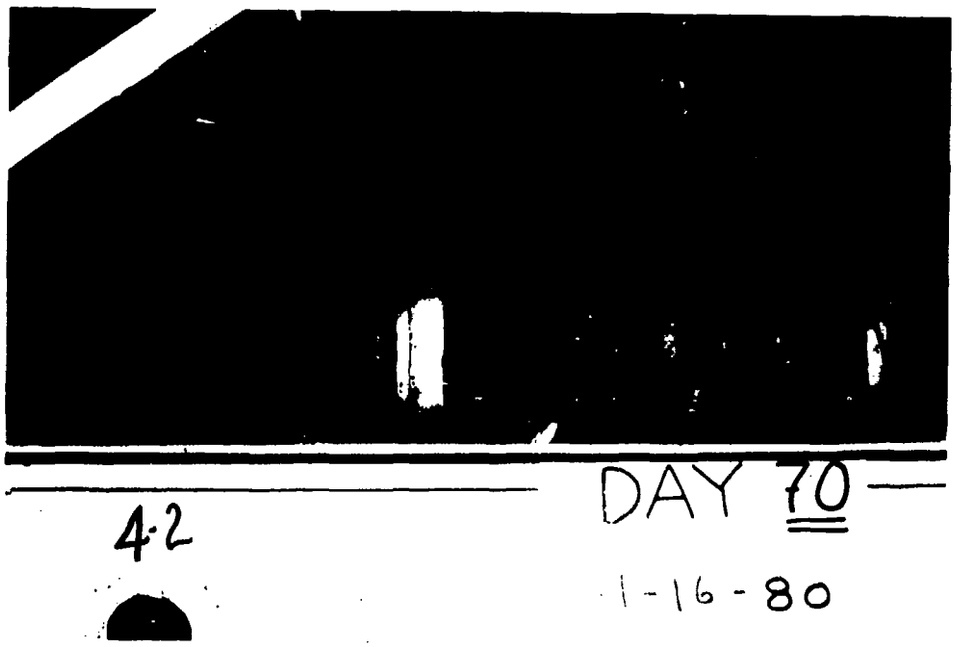


Figure B.6 Second Stage RBC Biofilms for the Calcium Hydroxide (Left) and Sodium Carbonate (Right) RBC Systems After 70 Days of Operation During the Alkaline Chemical Addition Study

DISTRIBUTION LIST

25 copies	Commander US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBG Fort Detrick Frederick, MD 21701
4 copies	USAMRDC (SGRD-RMS) Fort Detrick Frederick, MD 21701
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20014
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234
1 copy	Commander US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBD-A/Librarian Fort Detrick Frederick, MD 21701